Optimization of rearing techniques for cultured marine polychaetes (Nereis virens) using sustainably sourced ingredients.

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Optimization of rearing techniques for cultured marine polychaetes (*Nereis virens*) using sustainably sourced ingredients.

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Summary

The decrease in wild fish stocks has led to a search for novel feed sources to supply the aquaculture industry. Polychaetes have recently been identified as suitable feed ingredients due to their favourable nutritional composition, especially their lipid profile which is high in unsaturated fatty acids. As ragworm farms have started to develop, there has been a need to understand and improve rearing techniques, in particular the dietary requirements and nutritional profile of the ragworm.

In this thesis, research was focused on the king ragworm, *Nereis virens*. The protein and energy requirements were identified by increasing inclusion levels of nutrients in the diet as well as manipulating feed rations from starvation to satiation. Weight gain, survival, ingested feed as well as protein and energy retention efficiencies were evaluated. Results demonstrated that *N. virens* gained a proportional amount of nutrient in relation to the amount ingested up to a critical amount when gain either stagnated or decreased. A pattern of nutrient retention and maintenance requirements for different weight classes were calculated. This data was then used for bioenergetic modelling to calculate nutrient requirements using the equation:
Requirement: a x BW (g) + c x growth

The energy maintenance requirement was found to be 18 J g^{-1} worm day^{-1}; for protein, the requirement was 0.19 mg g^{-1} worm day^{-1}. The predicted weight gain (g) for a worm of any given size (g) was $y = 0.015g^{1.106}$. The total nutrient requirement is the sum of maintenance and growth, including the constant c which is the cost of nutrient deposition.

Alternative feed sources were also investigated to observe the extent at which *N. virens* can utilize novel sources and their abilities to preserve or convert highly unsaturated fatty acids such as EPA and DHA. Results showed a high adaptability to terrestrial animal or vegetable based feed sources but an inability to convert shorter chain or n-6 fatty acids when fed non-marine based feeds. There may however be potential for *N. virens* to utilize other feed sources from its natural environment to supplement n-3 HUFA content

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List of Abbreviations

ARA = arachidonic acid

ANOVA = Analysis of Variance

BW = body weight

cm = centimeter

C° = degree centigrade

DHA = docosahexaenoic acid

EFA = essential fatty acid

EPA = eicosapentaenoic acid

ECU = European currency unit

ERE = Energy Retention Efficiency

FCR = Feed Conversion Ratio

G = gauge

g = gram

h = hour

HUFA = Highly unsaturated fatty acid

ind = individual

J = joule

kJ = kilojoule

kcal = kilocalorie

kg = kilogram

Lab. = Laboratory

L:D = light:dark

Ltd. = Limited

LUX = Unit of illuminance

Med. = medium

ml = milliliter

mm = millimeter

mg = milligram

MJ = megajoule

n = number

PRE = Protein Retention Efficiency

PUFA = polyunsaturated fatty acid

rpm = revolutions per minute

R^2 = coefficient of determination

SGR = Specific Growth Rate

UK = United Kingdom

USA = United States of America

UVC = ultraviolet C

% = percent

‰ = part per thousand

" = inch

µm = micrometre

CHAPTER 1

General Introduction

1.1 Aquaculture Overview

The importance of the aquaculture industry worldwide had increased dramatically in the last few years and currently accounts for around 50 % of global food fish production (FAO, 2006). This industry is often described as a means to alleviate the loss of fish stocks which have occurred throughout many of the world's oceans. However, the industry has been beset by problems which have led to a large amount of negative publicity (Naylor et al., 2000) but are not always reflective of the true situation, as will be discussed in more detail in this introduction. The main problems have been related to pollution, disease, use of disease controls such as antibiotics and escaped animals which affect native species. All these factors affect the sustainability of the aquaculture industry and can lead to unfavourable overall perception of the industry by the public. It is important that aquaculture is perceived as environmentally friendly, especially today when sustainability and green concerns largely influence consumer choice. In response to these concerns, integrated multitrophic and organic aquaculture are on the rise (FAO, 2008). Another main aquaculture concern is the use of wild caught fish for use in fish meal and oils. The main focus of this section will be the use of aquaculture feeds, and how efforts are being made to reduce the inclusion of fish meals and oils in feeds in order to provide more sustainable feed options for the aquaculture industry.

1.1.1 The Need for Sustainable Aquaculture Feeds

A key problem facing aquaculture today is the use of fish meals and oils in feeds for farmed species. The main fish used are small, pelagic fish such as sardines, anchovy, herring, sand eel and sprat. These fish are mainly caught around the North Sea and around upwelling zones such as the west coast of South America. Many of the fish species are caught at or above their safe biological limit (Tacon and Metian, 2008). The occurrence of El Niño can also dramatically decrease levels of fish stocks; in 1996, global fish stocks decreased by 20 % due to the event (Pike, 1998). The global production of these species has however stabilized since 1985 from 6 to 7 million

tonnes (FAO, 2006) which implies an increase in competition for this resource, strongly suggesting other feeding methods will be needed. The FAO estimated that the contribution of aquaculture to overall fish production reached 47 % in 2006, with an average annual growth of 7 % from the early 1950's to 2006 (FAO, 2008).

Fish products can also come from by-catch resulting from fishing and fish trimmings from the processing industry. In 1997, one third of all fish caught was used for fish meal and oils destined for animal feeds (FAO, 1998). The economic benefit is obvious: by using low value species such as these, high value species such as sea bass, salmon, Mediterranean seabream and shrimp can be farmed. Nevertheless, there are many concerns about using these wild species as feed. First of all, continual fishing of these wild stocks is not sustainable; some areas have seen a severe depletion of once abundant species. Although Naylor et al., 2000, reported that aquaculture was contributing to the decline of fisheries worldwide and that the rise in aquaculture activities would further increase pressure on small pelagic fish stocks, fish meal production has changed relatively little in the last 15 years. The use of fish meal has been reallocated by market forces to aquaculture production over terrestrial animal farming (Tidwell and Allan, 2001). Secondly, there is a concern that taking these fish for aquaculture purposes is depriving low income communities that caught these fish for food. Furthermore, using these low value species to obtain high value species can lead to the latter being unaffordable for the poor.

At least 1 kg of fish meal is needed to produce 1 kg of farmed fish, (FAO, 2006) and 4 kg of wet fish are needed to produce 1 kg of dry fish meal (Allan et al., 2000). Fish based components such as proteins and lipids make up around 50 % of the feed. As these fish stocks become less sustainable, alternative feed ingredients need to be identified which would reduce the pressure put upon wild fish stocks. The price of fish meal has also been increasing due to stock depletion and continued demand for this resource, the International Monetary Fund estimates the price of fish meal to have risen from around $650/metric ton in 2005 to >$1000/metric ton in 2009.

In order for alternative feeds to be used on a commercial level preferentially to fish meal feeds, they need to be economical. The price of the feed ingredients has to be lower than fish meal diets currently available, the composition of the feeds must allow

for a healthy development, growth, survival and nutritional profile of the farmed species and they need to be either sourced sustainably or make use of wastes and by-products.

1.1.2 Variety of Aquaculture Feeds

The composition of aquaculture feeds is carefully formulated in order to satisfy the requirements of the target animals at different life stages, from larval feeds to maturation diets. The needs for proteins, lipids, fatty acids and other components vary and in order for an aquaculture operation to be successful which will ensure high survival, good growth, development of the body organs and healthy reproduction, the correct dietary requirements must be satisfied. Many diets focus on mirroring the natural diet of a species as closely as possible, which often currently include fish meals and oils. Marine based diets are usually highly successful as they contain classes of protein and lipids that are consumed in the wild. Increasingly research has focused on using high-protein plant sources as well as the by-products of animal rendering for feed.

The nutritional demands of carnivorous marine species limit the extent to which proteins from plants can be used. Suitable replacement levels in feeds have been investigated for many commercial fish species in order to decrease the inclusion level of marine protein sources. Many plant sources have potential to be used in aquaculture. Currently, the main species used are soy, wheat, lupin and rapeseed. The cost of fish meal compared to plant based meals is variable; for instance, the cost of plant based meals has recently risen due to an increase in the price of grain, following the relatively new and increased demand of plants for bioethanol. The increase in biofuel demand is estimated to account for 30% of the rise in weighted average grain prices (Rosegrant, 2008). Using plants as partial or total replacements for marine feeds has resulted in varying levels of success as many plant sources contain anti-nutritional factors. The main factors are protease inhibitors, lectins, saponins, phytic acid as well as many others which affect food utilisation and the health and production of the animals (Gatlin et al., 2007, Francis et al., 2001). Some anti-nutritional factors such as alkanoids also

affect the palatability of the feed. With advances in technology, anti-nutritional factors in plant feeds can be eliminated or reduced.

Waste products from meat industries have also been researched as alternative aquaculture feed sources (Bureau et al., 2000). The principal types of animal waste used are blood meal, meat and bone meal and in the poultry industry, feather meal. The disadvantage of using such feeds is the variable quality of the products; rendering and processing can affect the protein and nutritional content. High inclusions of meat or bone meal can lead to different results depending on the species; negative results such as low feed utilisation and growth rates have been reported for rainbow trout but positive results for gilthead seabream *Sparus aurata* (Robaina et al., 1997). There is also some apprehension over some human health aspects of using animal products; consumer concerns over issues such as bovine spongiform encephalitis (BSE) can restrict the use of such ingredients. Both plant and animal feeds also contain high levels of phosphorus which is indigestible (mainly from plants in the form of phytate); it passes through the gut and into the water column where it contributes to nutrient enrichment (Hardy and Tacon, 2002).

Feeds can also come in the form of yeasts or bacteria. Yeast by-products are natural additives which have shown to positively influence non-specific immune responses as well as growth in many fish species (Li and Gatlin, 2003).

1.1.3 Aquaculture Feed Proteins

Protein is the most expensive component of an aquaculture feed and traditionally has come from fish meal. Fish meal proteins provide a balanced essential amino acid profile which allows for high growth and survival of most farmed species. A majority of feeds given to aquaculture species have a predominance of proteins as they are essential for muscle development, growth, enzyme activity and cell function. Many plant based feeds contain lower protein levels and lower levels of essential amino acids than fish meals, although oilseed meals can contain much higher levels with 30-50 % protein (Halver, 2002). Protein sources derived from plants whether they are

oilseeds, pulses or grain are also known to lack essential amino acids compared with fish meal; the appeal of animal based feed lies in the high protein quality. Using vegetable sources can be expensive as prices continue to mount; added costs can also come from the addition of enzymes to counteract the antinutritional factors of many vegetable sources and supplementation of amino acids to improve the nutritional profile of the feeds (Tacon, 2004).

Proteins from soy or rapeseed can be processed and used in the form of concentrates. Gluten meal from soy, wheat or corn also has high levels of protein, between 60-75 % (Halver, 2002). Experiments have led to mixed results in marine fish trials. Complete substitution of fish meal can lead to reduced growth, perhaps due to lower palatability of the feed in seabream (Kissil et al., 2000) whereas partial substitution of fish meal can have satisfactory results in Atlantic salmon (Carter and Hauler, 2000). Herbivorous, carnivorous and omnivorous fish all require approximately the same amount of dietary protein per unit weight, yet herbivorous and omnivorous freshwater fish such as carp utilize plant based proteins better than carnivorous fish; the latter require minimal quantities of fish meal to supply essential amino acids (Naylor et al., 2000). The lack of high protein levels or suitable amino acid profiles in plant sources can be remediated to a certain extent by the inclusion of animal meals (Tacon and Jackson, 1985). Most terrestrial animal by-product meals, including poultry by-product meal, hydrolysed feather meal, blood meal, and meat and bone meal have high protein contents and favourable essential amino acid profiles (NRC, 1983).

1.1.4 Aquaculture Feed Lipids

Lipids play a very important part in the health, development and reproduction of marine aquaculture species. They not only provide energy for many different production processes, they also are the source of essential fatty acids (EFA) and are vital for the use of fat soluble vitamins. An EFA cannot be produced in the body and must be obtained from the diet; the EFA requirements vary amongst farmed aquaculture species. EFA

play important roles in cell synthesis, neural development, endocrine functions, reproduction and immune responses (Glencross, 2009).

Marine animals are rich in EFA, most notably arachidonic acid (ARA) 20:4n-6, eicosapentanoic acid (EPA) 20:5n-3 and docosahexaenoic acid (DHA) 22:6n-3. EPA and DHA are essential n-3 highly unsaturated fatty acids (HUFA) in fish where they play a role in maintaining the structural and functional integrity of cell membranes (Sargent et al., 1999). They also act as precursors to eicosanoids, a group of highly biologically active paracrine hormones, which are used in egg production, hatching, immunological responses and many other functions. HUFA also play an important role in cell membrane fluidity due to their very low melting points.

Important long chain n-3 HUFA cannot be found in significant amounts in terrestrial plants; they are more commonly found in fish and shellfish (Domergue et al., 2005), which results from the fatty acid composition of the marine phytoplankton at the bottom of the marine food chain. Marine species are characterised by low levels of linoleic acid (18:2 n-6) and linolenic acid (18:3 n-3) but high levels of long-chain n-3 PUFA (Steffens, 1997). Research done on marine carnivorous fish such as seabass, turbot and halibut show that these species have a limited, insufficiently fast or zero ability capacity to synthesize EPA and DHA from linolenic acid or arachidonic acid and hence require these fatty acids in their diet (Sargent et al., 1997). Farmed species are currently fed diets containing fish meals and oils and are therefore receiving the essential HUFA necessary to incorporate these essential fatty acids into the body and undergo healthy development and reproduction.

Larval stages of marine species are thought to be even more dependent on dietary HUFA as their high somatic growth rates cannot be fulfilled by their inabilities to synthesize fatty acids (Brett and Navarra, 1997). Juvenile marine fish require around 0.5 to 1 % of diet dry weight as n-3 HUFA (Sargent et al., 1993). DHA is particularly important at this stage (eg: for the development of the optical system in larvae) and feeds for larval and juvenile stages in aquaculture are often formulated to contain a higher ratio of DHA:EPA to satisfy their needs (Sargent et al., 1997). EFA are also important in maturation feeds; a maturation diet for penaeid shrimp leading to

successful spawning needs supplemental enrichment with HUFA, so that the fatty acids in the diet reflect the composition in the mature shrimp tissue.

Substituting plant oils for fish oils makes feed more sustainable, yet the class and quantity of fatty acids found in these oils is often unsuitable for marine species, notably n-6 fatty acids. Fish oils possess a low ratio of n-6: n-3 whereas vegetable oils have a higher n-6: n-3 ratio. The substitution of fish oils for cheaper plant oils leading to an accumulation of n-6 fatty acids in marine fish may not be favoured by consumers as human diets already have relatively high n-6: n-3 ratios. Humans are incapable of synthesizing *de novo* these fatty acids and must obtain them from their diet.

However, there are ways of using sustainable plant oils and retaining an adequate fatty acid profile. Feeding plant oil based diets to fish during their growing phase and then, a few months prior to slaughter, feeding a finishing diet containing fish oil, leads to a restoration of EPA and DHA levels in the body (Pickova and Mørkøre, 2007). The amount and duration of the finishing diet is dependent on species and growth rate.

Microalgae also show promise as a source of EFA. As previously mentioned microalgae form part of the plankton and are the basis of the marine food chain and the source of essential fatty acids. It is for this reason that microalgae species such as *Isochrysis* sp and *Chaetoceros* sp. are commonly used in aquaculture, especially the rearing of juveniles and larvae. Many mariculture species rely on the use of cultured microalgae, including oysters, abalone and prawns. Not only do microalgae contain high amounts of protein and lipid, but the amounts can also be altered by changes in temperature and aeration (Brown, 1997). Algae diets containing high levels of HUFA, especially EPA and DHA, led to an increased growth rate for oysters and scallops (Brown et al., 1997). Microalgae can be easily used in their natural form or processed into oils or spray dried preparations (Ward, 2005).

1.1.5 Polyculture

Another method of promoting a more sustainable aquaculture industry is using polyculture or integrated aquaculture (Milstein, 1992, Rothuis et al., 1998). This involves farming two or more species together, a mimic of natural systems in the wild and a technique used for centuries in Asia. Polyculture usually involves different species of herbivorous fish that utilize all available resources in a pond which is both economically viable and highly productive. Other integrated aquaculture systems involve molluscs and seaweeds which reduce nutrient and particulate loads from fish wastes. The waste from one species is recycled as the feed for another. Productivity is increased due to the sale of both the fish and additional farmed species; it is also environmentally friendly as waste loads are reduced. Many polychaetes such as *Nereis diversicolor* and *Sabella spallanzanii* are known to have bioremediating effects (Giangrande et al., 2005), and a considerable amount of research has looked at using polychaetes to both consume uneaten feed destined for fish or crustaceans and to filter waste products from the water column.

1.1.6 Bioenergetic Modelling in Aquaculture

In recent years, a growing aspect of research in aquaculture has been concerned with the use of mathematical approaches to quantify the nutrient requirements of farmed species with an ultimate goal of optimising feed formulations. This approach has been used extensively in terrestrial animals (Blaxter, 1989) and lately has been increasingly applied in marine animals (Lupatsch and Kissil, 2005). The general aim of the model is to identify the growth potential and nutritional composition of the animals at different weights and then coupling this with the sum of the nutritional requirements needed for both growth and maintenance. Based on these results, feed formulations can be deduced which will satisfy the needs of the animals of different weights.

In all living animals, the energy and protein ingested in feed is used for both maintenance and growth. Maintenance involves all metabolic processes that occur that

are needed for basic functioning of the body such as respiration, locomotion, osmoregulation and excretion. Any energy ingested into the body will be primarily used to satisfy these basic needs and any excess can be used for growth. The energy level fed which results in neither growth nor loss is known as the maintenance level. The maintenance level for protein is also important as any excess protein will be used as energy but primarily it is used for growth as well (Lupatsch et al., 2003, Bureau et al., 2006). Maintenance requirement of protein and energy provide a better understanding of the metabolic needs of the animal. This in turns allows for animals to be fed a minimum of nutrients in order to meet metabolic needs when they reach market size which reduces economic cost and prevents further growth.

Once the maintenance level for protein and energy has been determined the requirement for growth of the animals has to be identified. Ideally, the maximum growth potential when fed to satiation should be used as a measure to base those requirements on. In most cases, in absolute terms larger sized animals will show a tendency to put on more weight than smaller animals, resulting in increased feed demand. Similarly, higher feed consumption results in higher energy and protein intake which will result in higher gains of these nutrients in the body. All these related factors will provide set formulas from which the information used to quantify the nutritional requirements can be calculated.

1.2 Polychaete Farming

1.2.1 General Overview

Polychaete farming is a relatively new type of aquaculture industry, and one that has gained in importance ever since it first emerged in the 1980's. Various farms in the UK and abroad have been created; the species they concentrate on are *Nereis virens* and *Nereis diversicolor,* and to a lesser extent Arenicolidae, Eunicidae and other Nereidae. Polychaetes are farmed for two main purposes. The worms can be used live as bait for sea angling and as feed components in other aquaculture industries. Many live polychaete worms are imported into Europe from the Far East and the USA resulting in a substantial international trade. The trade is such that in certain areas there are more non indigenous imported species of worms sold as bait than native (Olive, 1999).

1.2.2 Bait Worms

In Europe, the bait worm market was estimated to be worth around 200 million ECU in 1999 (Olive, 1999), based mainly on worms being dug and collected from the wild. The advent of polychaete farming has led to the increased purchase of cultivated worms, leading in turn to a lesser dependence on wild bait digging which, in large amounts, can be destructive to the environment (Watson, 2007). Non target species may be affected, wading birds may be disturbed and their feeding grounds altered; habitat destruction also occurs in stable sheltered muddy areas where bait holes can remain for long periods of time (Fowler, 1999). There is also a risk of introducing an allochthonous species into a region, if polychaetes are harvested and shipped to areas where they are not native (Gambi et al., 1994). Farmed worms, especially nereids, have been increasingly used in bait shops and adopted by sea anglers resulting in a reduction of animals taken from the wild. The use of farmed worms for bait is predicted to rise as bait digging becomes increasingly perceived as a non sustainable activity (Olive, 1993). Economic factors will ultimately influence the success of farmed worms as bait as they

must be competitive in terms of price and quality relative to wild stocks (Olive, 1999). Farming of lugworm *Arenicola marina* (Olive, 1999) has also been investigated as it is another important bait species and patents on its rearing techniques have been produced. The majority of polychaete farms sell a high percentage of their worms as fresh, live bait; the worms can retain high quality and be sent long distances when packaged correctly in a moist environment.

1.2.3 Aquaculture Polychaetes

The establishment of many polychaete farms has led to the potential for further uses in the aquaculture industry. They are relatively easy to culture and possess high nutritional value; therefore farmed polychaetes make suitable candidates for aquaculture feeds. As many cultured finfish and crustaceans consume polychaetes naturally in the wild, the potential for their use as feeds is both feasible and environmentally sustainable. Bass, cod, flatfish and many other species of fish and crustaceans consume polychaetes in coastal zones during high tide or as part of a benthic diet. *N. diversicolor* was found to be around 45 % of the prey of flounder and 15 % of the prey of sole (Luis and Passos, 1995). Not only are polychaetes highly palatable to many marine species, they also contain high levels HUFA. The n3-C22 and n3-C20 classes of fatty acids are especially important in the development of finfish and crustacean juveniles (Olive, 1999). There is a potential for using not only polychaetes as maturation feeds for adult fish but also as a larval diet. Polychaetes have been considered to be the best maturation diets due their high levels of protein, lipids and HUFA (Meunpol et al., 2005). They also contain several hormonally active compounds (Lytle et al., 1990). However, there are some reservations over the use of wild polychaetes, among them the fluctuation of their nutritional value and possible disease transmission (eg: white spot syndrome virus) (Vijayan et al., 2005). The risk of disease transfer to shrimp when fed wild polychaetes can arise as polychaetes are bio-accumulators of heavy metals and other toxins (Rainbow et al., 2006). Heavy metals may be directly transmitted from feeds to ovary affecting nauplii quality. The controlled environment of a polychaete farm reduces this

risk greatly, which is important as there are currently no traceability systems with fresh feeds.

N. virens is chosen as a farmed polychaete species due to its controlled breeding technology, its mass production capability and adequate nutritional traits. In 2003, half of the production of *N. vi*rens was destined for aquaculture feeds (Pinon, 2003). As previously mentioned, one of the main concerns over the use of wild polychaetes as feed is their nutritional content variability; for example, the lipid content of *Nereis diversicolor* ranges from 6.6 to 19.3 % dry weight (Luis and Passos, 1995). The nutritional variability is due to the habitat, temperature, seasonality, the quantity and the quality of the food available, and the developmental and reproductive stage of the animal. When farmed, the controlled environment reduces the influences of these factors and the nutritional content is more stable. Olive, 1999, identified the criteria needed to farm polychaetes sustainably. They include developing procedures for the production of larvae and juveniles, broodstock and juvenile rearing, achieving year round supply by regulating the reproductive cycle and optimizing growth through control of nutrition, temperature and photoperiod.

1.2.4 Benefits of Polychaetes

Following the establishment of polychaete farms in the 1980's for the supply of live bait, the discovery of polychaete properties in relation to shrimp maturation development techniques added a further novel use for farmed polychaetes. Nutrients, in particular the n-3 and n-6 fatty acids found in polychaetes, are believed to be essential for the development of the prawn reproductive system (Lytle et al., 1990). *N. diversicolor*, the estuary ragworm, has been found to play a part as a nutrient stimulating gonad maturation and spawning in the hatchery reared common sole, in the Senegalese sole and in the penaeid shrimp *Penaeus kerathurus* (Luis and Passos, 1995).

Many reproductive hormones have been identified in polychaetes which make them known as an ideal feed for prawn broodstock maturation. These hormones include prostaglandin E2 (Meunpol et al., 2005), prostaglandin F2α (Poltana, 2005) and

vertebrate type steroids P4 and 17α-OHP4 (Meunpol et al., 2007). These hormones have a wide range of activities such as oocyte maturation, embryogenesis and vitellogenesis. Tirado et al., 1996, showed that by adding squid or polychaete in addition to a pelleted diet improved certain male characteristics in *Penaeus vannamei* such as spermatophore regeneration time. The levels of prostaglandins in farmed worms vary in relation to wild worms due to a feeding regime based on a fixed composition pelleted diet as opposed to a more variable wild one. Cultured worms are found to have less reproductive hormones due to the focus of the diet on DHA levels and lower intake of EPA and AHA relative to wild worms (Meunpol et al., 2005).

1.2.5 Lipid Content of Cultured Polychaetes

Nereids contain high levels of HUFA, especially n-3 fatty acids, which allows broodstock conditioning for egg production by crustaceans (Garcia-Alonzo et al., 2008). These high levels of HUFA make nereids a very suitable feed in aquaculture while alleviating pressure on wild fish stocks from which fish oils are currently sourced. Lytle et al., 1990, found that *N. virens* would provide an excellent balance of n-3 and n-6 fatty acids for a shrimp maturation diet. ARA was suggested by Middleditch et al. (1979) as a key HUFA in shrimp diets as it has been identified as a key fatty acid in shrimp gonadal tissue and as a precursor in the biosynthesis of prostaglandins.

Lipids in polychaetes function as a nutrient and energy reserve during periods of poor feeding and negative energy balance; these are usually non-polar or neutral lipids (Pocock et al., 1971). The structural lipids are constituents of biomembranes and include sterols and phospholipids. Marine invertebrates do not possess organs or adipose tissue for storage of lipids; polychaetes *A. marina* and *N. diversicolor* remove surplus fat from the gut, via blood or coelomocytes and store it in the epidermis. Samples of *Nereis* sp. polychaetes collected from Gilbert Bay, Labrador, had EPA and DHA levels of 24.4 and 3.5 % of fatty acids respectively and a total HUFA of 48.8 % (Copeman and Parrish, 2003).

The fatty acid profile of three different polychaete species *Glycera dibranchiate, Americonuphis reesei* and *N. virens* show an excellent balance of n-3/n-6 fatty acids for a maturation diet of *Penaeus vannemai* (Lytle et al., 1990). *N. virens* was found to have an n-3/n-6 of 12.6, EPA and DHA made up 71.6 % of the total n-3 fatty acids and n-3 fatty acids made up 39.4% of the total fatty acids.

Although the fatty acids in cultured *N. virens* have not yet been investigated, some studies have been performed on a close relative, *N. diversicolor*. The results from an investigation by Fidalgo e Costa et al., 2000 demonstrated that the majority of fatty acids were 16:0, 18:1n-9, 18:1n-7, 18:2n-6, 20:5n-3 and lesser amounts of 16:1n-7, 18:0, 18:3n-3, 20:2n-6 and 20:4n-6. The fatty acid composition in the worms reflected their diet. *N. diversicolor* was apparently able to biosynthesize *de novo* some fatty acids such as EPA and DHA when fed non or low fish based diets. Luis and Passos, 1995, suggested that diet could be the major single factor in the environment affecting lipid composition of *N. diversicolor*. Fatty acid composition is also influenced by gametogenesis and probably environmental temperature.

1.2.6 Production and Culturing of *N. virens*

In order for a ragworm farm to be successful and produce high quality animals, a number of considerations relating to the life history and habitat of the ragworm must be considered. The fact that *N. virens* is a semelparous species which dies after spawning makes the reproductive cycle a challenging obstacle for productivity. Through scientific research, the reproductive cycle has been controlled by adjustments of temperature and photoperiod. Worms were found to feed more under a longer photoperiod of L:D (light:dark) 16:8 instead of 8:16 (Last and Olive, 1999), thus inhibiting sexual maturation. During winter, the worms cease feeding and allocate energy to germ cells. By changing the photoperiod, the worms can be made to spawn or continue feeding and putting on weight depending on the needs of the farm. Photoperiodic changes stimulate the oocytes to develop; this is thought to involve enhanced rates of binding of the protein vitellogenin to the oocyte oolemma membrane

(Olive, 1999). Female *N. virens* will show an accelerated oocyte growth when photoperiod is changed from summer-like photoperiod of 16:8 to winter-like photoperiod of 8:16 (Djunaedi, 1995).

The response to photoperiod is however highly dependent on the age and growth history of the animal. Photoperiod can also affect segment additions; worms in long day photoperiod (L:D 16:8) regenerated lost segments better than worms kept in short day regimes (L:D 8:16) (Last and Olive, 1999). *N. virens* is shown to have a diel activity rhythm with nightly activity peaks. The general biomass of animals was a found to be larger in 16:8 L:D than in 8:16 L:D as all worms, even sexually immature specimens will reduce foraging activity when photoperiod is changed to a winter-like 8:16 (Last and Olive, 2004).

1.2.7 Ragworm Farms

There are two polychaete farms in the UK, Dragon Research Ltd. (Baglan, Wales) and Shoreline Polychaete Farms Seabait (Northumberland). They are situated close to sea and use pumped seawater as well as local beach sand for the worms to live in. The worms are housed in long shallow concrete raceways (110 m in length and 10 m in width) filled with around 15 cm of sand. Water is recirculated around the system using a paddle aerator. All raceways are housed outside which makes the ragworm susceptible to seasonal changes including temperature and salinity. Shoreline uses effluent hot water from a power station to keep beds at around 18°C throughout the year (Last and Olive, 1999) and produces around 60 tons of worms annually (Scaps, 2003).

Water temperature in the outdoor raceways as used by Dragon can fluctuate considerably, not only seasonal but also during the night. Temperature is found to affect the level of activity of worms; warmer water results in increased activity and feeding, while in cold water worms enter a more quiescent state of life (Deschênes et al., 2005). Shallow raceways can become very warm during summer months (>25°C) and high mortalities may ensue. Cold seasons are not as likely to cause mortalities; worms were found to survive even when covered in ice water (personal communication). However,

during cold periods the worms will feed less as a result of low metabolic rates. By keeping farmed ragworm at a constant temperature and fed at a demand rate, the ragworm become mature and subsequently die only during a few weeks of the year (Olive, 1999). Fertility of female *N. virens* is also very high and cryopreservation techniques have been developed in order to preserve up to a million larvae in order to ensure a steady supply of juveniles all year round (Wang and Olive, 2000). In the developmental stages, worms are stocked at around 1500 ind m^{-2} and the immature pre-adults harvested.

Although very resilient in both the wild and in farms, there are still some risks associated with culturing polychaetes. Worms can be predated upon by birds as the ponds are very shallow, although protective measures, such as netting covering the raceways, can be put into place. They can also be affected by pathogens such as *Vibrio* sp.; *Vibrio parahaemolyticus* is responsible for a disease in which the tentacles are destroyed and a gradual loss of sensory structures, abnormal behaviour and eventually mortality occurs (Scaps, 2003).

1.2.8 Polychaete use in Integrated Aquaculture

Polychaetes have been studied for their potential use as bioremediators for fish waste in aquaculture. All aquaculture operations result in waste, with a variable amount depending on the species and size of the animal as well as stocking density (Bischoff, 2007). Waste products can occur in the form of faecal matter or uneaten feed. Many waste removal strategies involve chemical, limnological or mechanical processes which can be complicated and expensive (Cho et al., 1994). The amount of waste and its negative effects on the surrounding environment can be mitigated to an extent by improving the efficiency of feeding methods as well as changing feed ingredients. Waste consisting of uneaten feed and faeces can accumulate in the environment surrounding the farm and seriously affect sediment chemistry and benthic communities (Carvahlo et al., 2007). The problem can be tackled through the use of polyculture involving combined mariculture and algae production, which can benefit from the waste; nutrients are recycled which not only alleviates pollution problems but also can

generate increase production value for the farmer. The idea behind this method is that one species' waste is another one's feed; systems are set up involving many trophic levels reflecting natural ecosystems in the wild.

Many polychaetes such as *N. diversicolor* have been identified as helpful in controlling pollution: they consume organics in the sediment and their burrowing increases oxygen content (Kristensen, 2001). *N. diversicolor*, in individuals of comparable size, has no conspicuous morphological differences to *N. virens*. They differ mainly in feeding modes as *N. diversicolor* is a facultative filter feeder (Nielsen et al., 1995). Experimental trials were set up in Japan using *Neanthes japonica* and *Perinereis nuntia* for the treatment of domestic sewage. The study found that the system would not be able to function in highly populated areas as the necessary worm biomass would be too great but it may be of use in smaller towns (Kurihara, 1983). Trials have also been made with gilthead sea bream *Sparus aurata* feed and *N. diversicolor*, the idea being that the polychaete worm would consume uneaten sea bream pellets (Batista et al., 2003). The worms were found to be able to survive, develop and reproduce on this diet which shows the potential for polyculture with *S. aurata*.

1.3 King Ragworm *Nereis virens*

1.3.1 Physiology of *N. virens*

N. virens is a member of the Nereidae, Order Phyllodocida. It is also known as the sand worm or clamworm. Body length can reach up to a metre but usually attains around 20-40 cm at maturity. They are segmented worms, each segment containing a pair of parapodia with bristled chaetae. The segmented body is composed of a prostomium at the anterior end and a posterior pygidium. The large parapodia are used for swimming and gas exchange. Although *N. virens* spends most of its time burrowed, males exhibit swimming activity during reproduction. The gut is similar to most invertebrates in that it possesses an ectodermal foregut, an endodermal midgut and an ectodermal hindgut. The gut is a long specialized tube which begins at the mouth and ends at the anus on the pygidium running the length of the body of the worm. The head of the worm bears two palps, two tentacles and four pairs of tentacular cirri which the worm uses to sense its environment and neighbouring worms. It also possesses four eyes and chemosensory nuchal organs. The mouth is located beneath the prostomium and contains a pharynx which becomes everted when feeding. This large pharynx bears pragnaths which are used to capture food particles. The pharynx also bears two black chitinous jaws which are also used for feeding.

1.3.2 Ecology of *N. virens*

N. virens is an estuarine polychaete found in temperate shallow marine soft-bottom coastal habitats. It is also found in other environments of the intertidal zone such as mussel beds and salt marsh root systems (Pettibone, 1963). It is mainly sublittoral, but can also be found near the low tide level exposed to emergence for one or two hours (Schöttler, 1979). The distribution of the worms is highly dependent on the age of the worms as juveniles and adults are spatially separated. This species can be found in temperate regions across the Northern Hemisphere. It is very eurythermal and

euryhaline which is an adaptation to estuarine life as temperatures and salinities can fluctuate widely with tidal variations and seasons. This species is found on both sides of the Atlantic and as far down as France and although tolerant, is rarely found at salinities below 15 ‰ (Nielsen et al., 1995).

1.3.3 *N. virens* Lifecycle

Nereis virens has a semelparous life cycle; it reproduces once and then dies. Like many polychaetes it lacks permanent gonads; immature gametes are formed from cells in the ventral peritoneum and released into the coelom where they complete their maturation. Energy invested in growth becomes used for reproduction processes (Olive et al., 1997). This reproductive period culminates in mature animals in springtime, usually around April or May. Age at maturity is highly dependent on the size of the animals. High growth rates lead to an early onset of maturity, but lower growth rates and greater age at maturity result in a smaller size at maturity (Desrosiers et al., 1994). Age is not very significant in terms of time of maturity as it can occur between 1 and 8 years (Brafield and Chapman, 1967).

Adults reach sexual maturity in the spring, around April and May, at which point the coelomic fluid of the separate sexes is filled with maturing oocytes and sperm. The worms feed more in the summer in order to build up energy reserves, which may then be transferred to the developing oocytes the following winter. During the time in which energy is being processed, feeding activity is suppressed because reproduction is followed by death and therefore energy acquired late cannot contribute to future fitness; also foraging would increase chance of predation (Last and Olive, 1999).

During the period of maturation, the worms cease to feed, undergo histolysis of both body wall and gut tissue and change in colour. Ripe females become a vivid emerald green with bright green eggs visible in the parapodia and the coelom, while mature males become milky green due to the presence of sperm filling their coelom and immature worms have extensive peripheral vascularization and yellow, fleshy parapodia (Pocock et al., 1971).

N. virens, like many other nereids, show swarming at night. Gravid males leave their burrows, when in the water column; they swarm before releasing their gametes. The females apparently stay in their burrow in order to avoid predation (Chatelain et al., 2008). Males undergo epitoky, physical changes that occur in preparation for reproduction. The epitoke is a modified state for reproduction and is characterized by sensory and locomotory system changes. The changes include enlarged parapodia, natatory chaetae, gut atrophy, histolysis of the body wall and reorganization of musculature (Chatelain et al., 2008). Body reserves are used up as energy for the formation of gametes. Females may perhaps continue feeding during maturation as they stay in their burrows. In either case, both sexes die following spawning. Death occurs due the considerable damage sustained by the body wall during spawning (Bass and Bradfield, 1972). Fertilization occurs in the sea and results in a larva which stays close to the substratum for around 5 or 6 days. There is then a short planktonic phase called a trochophore which lasts around 15 hours. The period may be brief in order to prevent the trochophores from drifting into unsuitable areas (Bass and Bradfield, 1972). Trochophores then become nectochaetes which both swim and burrow in the sediment. Eventually, generally after the twelfth day after fertilization, they become truly benthic.

Populations of juvenile and sexually mature worms are spatially separated. Immature individuals inhabit the upper intertidal, whereas mature worms are found further down the shore. This arrangement is due to the recruitment of larvae into the upper tidal zones and to the migration of adults downshore (Miron et al., 1992).

Growth of the worms was investigated by Bass and Bradfield, 1972, who estimated that the young worms reach 0.2 to 1 g within 13 to 16 weeks after spawning. The worms can then reach 2 to 3 g after 1 year and 17 to 18 g at around 2 years. Olivier et al., 1996, found that recruits had a specific growth rate of 0.4 to 1.7 % d^{-1}. There can be inter and intra-specific variations in growth rate which can be due to differences in behaviour, genetic variation and species life span.

1.3.4 Feeding Mechanisms of *N. virens*

In the wild, *N. virens* adopts a variety of feeding strategies. Estuarine animals live under the influence of tides, seasons and fluctuating, conditions that compel it to have an adaptable diet. Food supplies can be unpredictable and erratic and *N. virens* possesses different feeding modes and physiological adaptations to survive.

The anatomy of the feeding structures has been well documented and aid in establishing feeding modes. The morphology of the jaw consists of feeding appendages at the mouth of the worm and noted eversible pharynges, often adorned with small auxiliary jaw pieces called pragnaths. *N. virens* feeds by capturing a food item with its eversible proboscis armed with two curved jaws bearing 5 to 10 chitinous teeth. The jaws are hardened, especially the distal portions, with zinc (2.4 % of total jaw weight) (Wilson, 1988). The gut of *N. virens* contains striated spines which apparently triturate food items (Michel and Devillez, 1980). *N. virens* feeds by extending a portion of its body from an opening of its mucus lined burrow (Wilson and Ruff, 1988).

Lewis and Whitney, 1968, noted that cellulase does occur in *N. virens*, produced solely by the gut, and its production is induced by the presence of algae. Chapman and Taylor, 1968, discovered that *N. virens* was able to uptake amino acids and other small organic molecules from the surrounding water. It can take up a range of organic compounds including not only a variety of amino acids, but also succinic, glycolic and citric acids and glucose. *N. virens* is also able to absorb amino acids from seawater in which they are present at only low concentrations (Taylor, 1969).

Enzyme activities to establish *N. virens* diet were investigated by Bock and Mayer, 1999. Lipase to protease ratio was found to be a useful tool in comparing enzyme activities. In carnivorous polychaetes the lipase:protease ratio was normally over 1 and in detritivores less than 0.04. They looked at *N. virens* to discover whether an omnivore maintained on a carnivorous versus detritivorous diets adjusts its enzyme activities accordingly. *N. virens* was fed on kelp, sediment and mussel. It showed considerable plasticity in its enzyme activities: carbohydrate hydrolyzing enzymes showed little variation with diet, but both protease and lipase activities varied from levels observed previously for detritivorous and carnivorous polychaetes.

Lipase/protease ratios placed this species as intermediate between detritivores and carnivores. This implies significant plasticity in the luminal enzyme activities of this omnivore; it changes its digestive chemistry in response to its diet.

1.3.5 *N. virens* Feeding Strategies

Studies on *N. virens* have revealed a range of feeding modes. Pocock et al., 1971, suggested that in their natural habitat, the worms ingested mud, and presumably derived their nutrients from particulate organic material contained within. Olivier et al., 1995, discovered that *N. virens* consumed decaying algal matter on the shore with high nutritive value that is augmented by the presence of bacterial colonies. Other studies have found *N. virens* to be a facultative detrital feeder (Tenore and Gopalan, 1974). A study by Neuhoff, 1979, found that when fed oyster biodeposits and clam tissue, although the clam tissue was a more nutritive source, the growth of worms fed on biodeposits compared favourably with those in nature. Papaspyrou et al., 2006, described the species as being an obligate deposit feeder.

Fauchald and Jumars, 1979, depicted the species as being more of an omnivore. Kay and Bradfield, 1973, observed that *N. virens* ingested both animal and plant material. This shows an excellent strategy for adaptation in an unstable and changing environment such as the intertidal zone. Lewis and Whitney, 1968, found evidence of this theory in a study of the gut content of the ragworm. An analysis of the gut of *N. virens* showed gastropod shells (*Hydrobia*) small cockle (*Cardium edule*) and algae *Enteromorpha intestinalis*. In laboratory experiments, the worms readily ate dead mussels, cockles and shore crabs. When fed *E. intestinalis*, undigested strands of algae were observed in the faeces. Caron et al., 2004, found a variety of food items in the gut of *N. virens* (which is also the main predator of *Macoma balthica* in Quebec): vegetal and animal detritus, diatoms, *N. virens* appendages, crustaceans, gastropods, foraminiferans, nematodes, hydrozoans and other polychaetes. He suggested that *N. virens* seems to be opportunistic feeder and this is probably related to the natural fluctuations of its food source in a littoral ecosystem.

1.3.6 Feeding and Life Stage

Different feeding strategies have been noted depending on the various life stages of *N. virens*. Olivier et al., 1996, established that adults were omnivorous and opportunistic, using many feeding modes such as deposit feeding, scavenging and predation. The worms seemed to be actively involved in the transfer and integration processes of particulate matter throughout the benthic community. On the other hand, juveniles feed mainly on degraded and partly degraded plant detritus. The study focused on feeding different plant materials to juveniles and found that weight specific growth rate varied from 0.4 to 1.7 % d^{-1} for individuals fed a range of marine vascular and algal plant sources. A higher weight specific growth rate occurred for individuals fed on algae such as *E. intestinalis*, *Laminaria longicruris, Fucus vesiculosis* (higher caloric content and higher net production/food intake) in comparison to high lignin haplophytes such as *Zostera marina* and *Spartina alterniflora*.

Lignin in vascular plants is a barrier and renders non decomposed vascular plants unavailable to many macroconsumers. Juveniles mainly inhabit the organic rich upper portion of the sediment (0-12 cm) while adults colonized greater depths (>25 cm) where organic matter content was lower. A number of specific mechanisms are present enabling the worms to absorb a range of compounds simultaneously, such as amino acids in seawater. However, it seems that the uptake is more efficient for young worms. These juveniles are probably deriving nutrient from their environment in this soluble form, although as their ability to synthesize food becomes more developed, the absorptive route may become less important.

1.3.7 Feeding in Relation to the Environment

The estuarine habitat, fluctuating tides and burrowing behaviour require *N. virens* to maximise their food usage. Deschenes et al., 2005 noted that food is exported from the shores and into the subtidal zone by ebb tides and imported by flood tides.

Therefore, food is available for only short periods every day, especially since *N. virens* are more active by night. *N. virens* may hence avoid temporary food shortage by accumulating organic matter in their burrows; *N. virens* stores its food by imbedding it into the burrow wall (Deschenes et al., 2003).

Polychaetes have slower intestinal transit in colder waters, which results in higher absorption efficiency. When food was abundant and at high temperature, *N. virens* appeared to store food immediately, enabling it to acquire larger food reserves in a short time span. Hoarding large amounts of algae at high temperatures suggest short term storage because of high food degradability, whereas hoarding under colder temperatures suggests longer term storage because of low food degradability. Moreover, stored food was usually ingested between observation periods at high temperatures and was left untouched at low temperatures. Using *E. intestinalis* as a food source, it was observed that relative activity in response to food stimuli was controlled foremost by temperature.

It is also thought that *N. virens* lines its burrow with mucus in order to trap phytoplankton; however this behaviour is more pronounced and common in its close relative *N. diversicolor*. The latter species not only has a more developed burrow than *N. virens*, it also irrigates the burrow between 5 and 40 times more than *N. virens* (Papaspyrou et al., 2006). From the concentration of microbenthos on the burrow wall, it also seems likely that *N. virens* is unable to exploit this resource.

This hoarding behaviour exhibited by *N. virens* may be explained by the species anticipating short or long term food shortage (winter time). Some vascular species also require decomposition before acquiring sufficient nutritional value and may be stored for longer periods (Deschenes et al., 2003, Olivier et al., 1995); this behaviour is reminiscent of the "gardening" displayed by the polychaete *Abarenicola pacifica* which cultivates bacteria to ensure a constant food supply (Hylleberg, 1975).

1.4 Aims and Objectives

The overall aim of this research is to optimise feeds and feeding regimes for the production of *N. virens.* The primary objective is to quantify the energy and protein requirements of *N. virens* in order to formulate optimal feeds that yield high performance and low waste. The ability to retain protein and energy throughout different weights and life stages will allow a prediction of prospective production and optimization of growth for *N. virens*.

The secondary objective is to assess the ability of *N. virens* to utilise feeds made from non-marine raw materials, such as plant sources, wastes and by-products, while retaining high nutritional composition. This will include quantification of the essential fatty acid composition of *N. virens* in relation to feed composition.

Knowing the extent and potential use of formulated feeds containing sustainably sourced raw materials and the use of nutrients by a wide range of worm sizes will allow for more sustainable culturing of *N. virens* and a more precise and less wasteful feed delivery.

CHAPTER 2

General Materials and Methods

2.1 Experimental Setup

2.1.1 Wet Laboratory

All experiments were performed at the Swansea University School of the Environment and Society research facilities. The main facility used was a wet laboratory consisting of multiple raised benches receiving recirculated seawater. The seawater was stored in a 40 m^3 underground sump and supplied to the benches under pumped pressure. The seawater was pumped directly from the sea and was unfiltered and unsterilized. Approximately 10% of the sump capacity was replenished twice per week with new filtered seawater supplied from the adjacent Centre for Sustainable Aquaculture Research (CSAR). A chilling system enabled water temperature to be maintained below ambient temperature in the wet laboratory (necessary during summer). Light within the laboratory was provided by 400 LUX strip lights with a photoperiod L:D of 16:8. Temperature and salinity were monitored regularly using an Oxyguard hand held probe.

2.1.2 Sediment

Sand used in all experiments was taken from the high water mark on Swansea beach. Sediment particle size analysis was performed using the sand sieve analysis method and data plotted using Phi particle size values (Fig. 1). The sediment consisted of a large majority of Φ=2, classifying this sediment as fine sand. Prior to each experiment, sand was roughly sieved for macrofauna using a 2 mm mesh sieve.

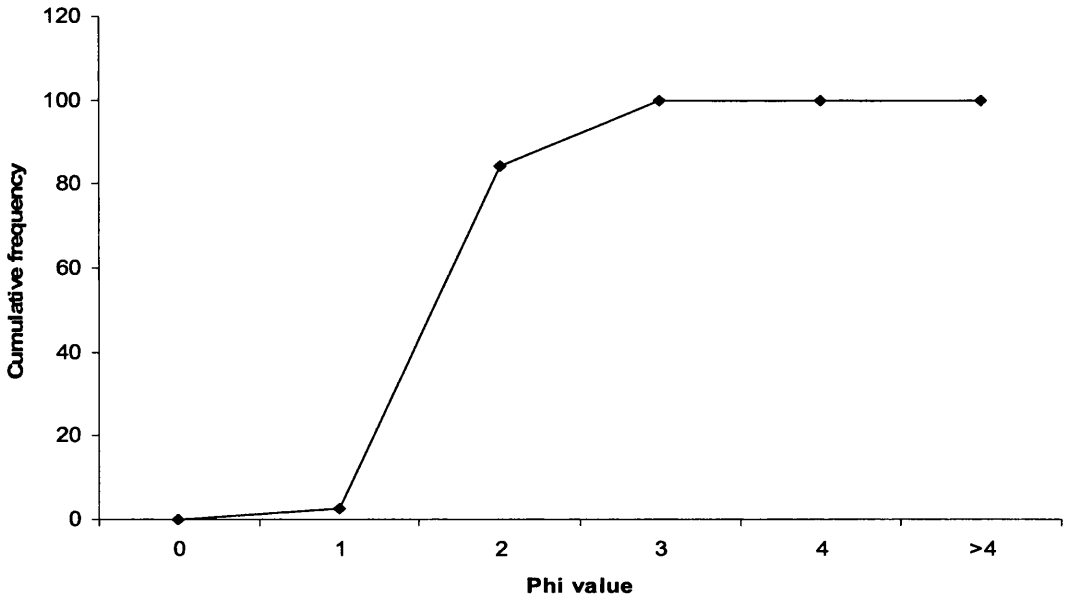

Fig. 1. Cumulative frequency curve of sediment taken from the High Water Mark on Swansea Beach.

2.1.3 Rearing Containers

Clear polypropylene plastic tubs measuring 42 x 35 x 26 cm were used in all experiments, including experiments in which individual, smaller pots were used within tubs (described in Materials and Methods, Chapter 3). Each tub was filled with sand up to a height of 10 cm, sufficient for 12 to 15 worms to burrow. The tubs were placed on the bench and each tub received an individual water source from outlets positioned above the bench. Water flow into the tubs was constant. Excess seawater flowed out through sides of the tubs over which 0.5 mm mesh was placed to prevent worms escaping.

2.1.4 Water Analysis

Water temperature and salinity were monitored regularly (approximately three times per week). Nitrite, nitrate, ammonia, pH and alkalinity were measured regularly by colorimetry test kits (Hach, Germany). Water chemistry was carried out during the duration of the experiments and in particular when new water was pumped in or other livestock from different research projects introduced into the laboratory.

2.2 *N. virens* Sampling, Measurement and Monitoring

All experimental *N. virens* originated from Dragon Research Ltd. in Baglan. Harvested individuals at the farm were placed in sediment-free water-filled troughs and individual animals were size graded by hand, transported back to the laboratory in cool boxes before being prepared for each experiment. Individual worms were briefly blotted dry on paper towels before being weighed (all weights taken to .00 g) and released into their tubs. Three replicates of 10 initial worm samples were briefly rinsed in freshwater (2-3 seconds) to remove salts, blotted dry on paper towels, weighed, bagged and frozen at -80°C until further analysis. Takedown of experiments also followed this procedure; all worms were rinsed, dried, weighed and frozen until further use.

Body weight (g) was employed instead of length or segment number as many studies have found weight measurement to be more accurate (Dean, 1978). *N. virens* can easily expand and contract the body longitudinally and segments are very often lost from the posterior end of the animal. Prior to the start of each experiment, the worms were left to acclimatise to their new habitat for 24 hours. At the end of the experiment, worms were removed from the sand and placed in clean seawater for 2 days to purge of any sand (or feed) remaining in the body. Purging was an essential step to not only obtain accurate body weight measurements relative to the original size (worms taken from the farm had also been purged for a certain time) but also to produce consistent and accurate results during the carcass analysis. During the length of each experiment

any dead or dying worms appearing on the surface of the sand were removed and enumerated daily.

2.3 Feed Manufacture

Feeds for the experiments were mainly manufactured at the Dragon Research Ltd. Premises in Baglan. The feed was produced using a Baker Perkins MPF 50 twin screw extruder. The extruder processes powdered ingredients, oils and water at 85°C and was able to deliver up to 100 kg $hour^{-1}$. The resulting mixture was fed through a die; the most appropriate size for worms being 1-2 mm diameter. Small pellets were then chopped with a knife before being dried in a fluidised bed drier for 25 minutes, leaving the pellets with a moisture content of around 8 %. One feed, a commercial fish meal based diet destined for trout and manufactured by Skretting, was used at Dragon Research Ltd. as feed for the worms in the raceways during a portion of the current research and also used in experiments, mainly as a control.

2.3.1 Feeding Regime

Feed destined for each tub was weighed out daily and sprinkled by hand evenly into the tub in the morning. Worms were left to consume the feed over 24 hours. Leftover feed was accounted for by individually counting remaining pellets; the average weight of individual pellets was determined prior to the experiment (in the case of large amounts of pellets forming an indistinguishable mass, an estimation of numbers was taken using the density of pellets present). Any remains were siphoned out and discarded. Consumed feed was calculated as the number of pellets given minus the number of pellets remaining.

2.4 Analysis

2.4.1 Dry Matter and Ash Content of Sand

Duplicate sand samples of around 15-30 g were taken from each tub, bagged and frozen at the end of each experiment. Sand samples were removed after a thorough mixing of the sediment in order to obtain homogenous replicates across the experiment. The samples were then allowed to defrost before being placed in foil trays in an oven at 105°C for 24 hours to obtain dry weight. Duplicate samples were placed in ceramic crucibles and combusted in a Carbolite 201 muffle furnace at 550°C for 12 hours for ash content measurements.

2.4.2 Dry Matter and Ash Content of Ragworm and Feed

Dry matter of feed and ragworm was calculated by weight loss after 24 h drying in an oven at 105°C. The water content was calculated by subtracting the end weight after drying from the original weight. Dried samples were then homogenized in a coffee grinder prior to all further analyses. Ash was calculated from the weight loss after incineration of the samples for 12 h at 550°C in a Carbolite 201 muffle furnace. Pelleted feed was ground to a fine powder before being analyzed in the same manner as the worm samples.

2.4.3 Chemical Analysis

All chemical analyses were carried out by a trained member of staff using the following protocols for energy, protein, lipid and fatty acid composition.

2.4.3.1 Energy Analysis

Gross energy content was determined using a Parr bomb calorimeter using benzoic acid as a standard. A sample of dried, blended ragworm was used in duplicate.

Less than 1 g of sample was used per run. The sample was weighed and placed in a dish inside the bomb calorimeter as well as 1 ml of water placed at the bottom of the bomb and 10 cm of ignition wire connected to the terminals inside the bomb. The bomb was then flushed twice with 10 atm oxygen, then filled to a pressure of 25 atm. 2000 ml of distilled water was then added. After 5 minutes, 5 temperature readings were taken with 1 minute intervals. Following the firing of the compound, the firing switch was opened. Temperature readings were then taken every 30 seconds for 5 minutes.

The heat of combustion at constant volume ($-\Delta U$ (cal/g) was determined using the equation: $-\Delta U = (tW - e_1 - e_2) / m$

W = heat capacity of the calorimeter

e_1 = correction for the heat from the formation of nitric acid

e_1 = correction for the heat from the combustion of the wire

m = mass of the sample

2.4.3.2 Protein Analysis

Protein analysis was performed using the Kjeldahl technique. A weighed (around 1 g) sample was placed in a digestion flask with 12-15 ml concentrated sulphuric acid, potassium sulphate and a catalyst. The mixture was brought to the boil for an hour and then cooled. Sodium hydroxide was then added to raise the pH and the mixture boiled again, distilled vapours were trapped in a solution of 15 ml hydrochloric acid and water. An indicator dye was added to the trapping solution along with sodium hydroxide. The amount of sodium hydroxide needed for the solution to turn orange was recorded.

The following calculation was used to obtain nitrogen estimates:

Nitrogen g = moles nitrogen x atomic mass of nitrogen

moles nitrogen = calculated by subtracting the moles of base used to neutralize the solution by the number of moles trapped in the flask originally.

A further conversion into % Nitrogen x 6.25 gave the level of crude protein in the sample.

2.4.3.3 Lipid Analysis

The samples were homogenized with 17 times the volume of solvent to tissue with 2:1 chloroform. The material was then poured through a scintered glass filter and allowed to drip through. The tissue was then washed with 3 times the volume as originally homogenized giving a final volume of solvent of 20 times the tissue sample. The sample was then poured into a stoppered graduated cylinder after which 0.20 times the volume of the sample was added as water, shaken and left to stand overnight to separate. The volume of lipid containing solvent phase was recorded the next day. The upper phase was removed with a vacuum and the sides of the graduated cylinder washed with 10-20 ml of new upper phase solvent with the following composition:

Upper phase solvent = chl:meth:water = 30:480:470

The known amount of solvent containing the extracted lipids was then removed and evaporated under a stream of nitrogen After evaporation, the sample was put into a dessicator under vacuum overnight to remove any moisture

2.4.3.4 Fatty Acid Analysis

The fatty acid analysis was carried out using the methylation procedure. Using the extracted lipid sample, 40 mg of lipid was removed into a stoppered test tube and 100 µl of the 17: 0 standard was added. The sample was then evaporated under nitrogen at 50°C in a water bath. 2 ml 1 % sulfuric acid in methanol and 0.5 ml chloroform was added to the test tube of nitrogen evaporated lipid, shaken and added to a 50° C water bath overnight. To methylated esters, 4 ml of water and + 2 ml 2% $KHCO_3$ were added and the sample was stirred. 5 ml of hexane was then added, stirred and allowed to separate, after which the upper phase was removed. The extraction was repeated from the remaining lower phase using 5 ml 1:1 hexane:diethyl ether and combined with the first extraction. 5 ml of water was then added, stirred and allowed to separate, after which the upper phase was removed to a test tube. The methylated esters were nitrogen

evaporated and re-dissolved in 1-2 ml hexane for injection into the gas chromatographer. After the injection, the amount of fatty acids was calculated in mg/100 mg dry sample using the following procedure:

$$\frac{(\text{Factor of 17:0}) \times (\text{total amount of solvent}) \times 100}{(\text{Area \% of 17:0}) \times (\text{amount solvent for methyl}) \times (\text{weight of sample in mg})} \times \text{area \% of fatty acid}$$

2.5 Data Analysis

2.5.1 Experimental Design and Statistical Analysis

Replication was achieved in experiments via multiple (2 to 3) tubs of worms per treatment, with up to 5 diet treatments being tested simultaneously. The tubs were numbered and laid out randomly in the wet laboratory. Stocking density was established at approximately 12 to 20 worms per tub depending on the starting size required for the experiment. All parameters were expressed and analysed as mean value per tub.

Statistical Package for Social Scientists 13.0 (SPSS) was employed for all statistical analysis. Means and standard deviations were calculated for data points and Kolmogorov-Smirnov normality tests were performed; Shapiro-Wilk normality tests were used for smaller data sets. All percentage data was arcsine transformed before analysis. A one-way analysis of variance (ANOVA) determined differences within treatments when only one variable was concerned, followed by a post-hoc Tukey's test to evaluate the level of these differences. For experiments in which two variables were tested, such as different habitats and feeds, a two-way ANOVA was performed. Linear regression was also employed to establish any relationships between two variables. Polynomial contrasts were used to perform non-linear curve fitting or curvilinear regression, using individual tub replicates pooled from multiple experiments.

2.5.2 Mathematical Formulae

Body composition and performance parameters were calculated using the following equations:

Feed Conversion Ratio (FCR) = Feed Consumed / Weight gain

Specific Growth Rate (SGR) = (Ln Weight Final– Ln Weight Initial)/ Days * 100

Geometric Mean Weight = (Weight Initial * Weight Final) ^ 0.5
(due to non-linear growth of the worms, the geometric mean was used)

% Feed Intake per day per worm = (Feed Consumed / days) / (Weight final * Weight initial) ^ 0.5) * 100

Protein Retention Efficiency = Protein Gain / Protein Fed * 100

Energy Retention Efficiency = Energy Gain / Energy Fed * 100

CHAPTER 3

Quantification of Feed Requirements for Nutrient Retention and Growth of *N. virens*.

3.1 Introduction

A primary consideration when investigating the feed requirements for *N. virens* is to assess the potential of the worms to grow and retain protein and energy in the body. A comprehensive estimation of feed requirements requires the creation of a feed budget model which will allow for a precise feed delivery with a tailored composition. Feed budget models can be based on growth rate or energy requirement estimates. However, in practice polychaete farmers use more arbitrary adjustments without accounting for variables like temperature, growth, body composition, season which may influence the growth period of the animal. Furthermore, there is no indication of the amount of feed consumed due to low visibility in the water. The guidelines on feeding cultured polychaetes are currently vague and developed mainly to establish high growth and survival; the effects of different feed levels on *N. virens* nutrient retention and body composition are hence largely unknown.

It is necessary to identify the most appropriate feeding levels for the growth and physiology of the worms, but also for environmental and economic reasons. Feeding a surplus to requirement of feeds would lead to a build up of nutrients in the pond which will eventually lead to anoxia of the sediment and water and an increase in bacteria. This excess of feed can also affect the industry economically as feed is one of the most expensive elements of polychaete production. Finding an appropriate feeding level would reduce feed expenses and resource waste while maintaining a cleaner environment for the worms to live in.

Coupled with feeding ration investigations is the need to research the nutritional requirements of *N. virens*, especially protein and energy, which would aid significantly in devising suitable feed formulations. In the wild, *N. virens* is an opportunistic omnivore and detritivore relying on food brought in with the tide and estuarine fauna and flora; this implies a highly variable intake of protein and energy; this species can also act as a predatory carnivore on other infaunal animals. This ability of *N. virens* to utilise various feed sources is well known, yet feeding strategies employed in the wild

do not necessarily translate as high growth and nutrient retention which is of vital importance in polychaete farming.

A factor which greatly influences feed intake is maturity of the animal; *N. virens*, being a semelparous species which spawn once during their lifetime and subsequently die, also cease feeding in their reproductive phase as they reach maturity (Last and Olive, 1999). At the commercial sponsor site, worms are harvested as immature adults (personal communication), therefore this issue should not present a significant problem.

The aim of this chapter was to identify a suitable nutrient intake via the feed which results in not only high growth but also high energy and protein retention efficiency within the body, as a basis for future bioenergetic modelling of the feeding requirements of *N. virens*. A range of worm sizes were used to obtain a large data set on general ragworm growth as well as feeding behaviour and body composition. Not only can the growth of the animals be assessed in relation to the amount of feed given, the efficiency of nutrient utilization from feeds such as protein and lipids can also be evaluated.

The effects of withholding food from worms, with the associated weight decrease and loss of nutrients were also assessed. By conducting starvation studies with the worms, the minimal metabolic or energetic requirements could be measured by looking at the relative loss of a certain nutrient in the body. The starvation studies also established whether *N. virens* may have utilised any feed sources in the surrounding water or sediment within the experimental design which may have compromised results.

3.2 Materials and Methods

3.2.1 Experiment 1: Effects of Increasing Feed Rations – Medium Sized Worms

The feed intake, growth and nutrient retention of individual worms were observed in this experiment. Plastic plant pots, 17 cm in diameter, were positioned into plastic tubs; each tub contained 6 pots, each housing one worm. Each pot was surrounded by 500 μm mesh which rose over the water level to prevent escapees but to allow water flow. Sieved sand (approximately 1000 g) was added to each pot. Mean body wet weight of worms used was 4.5 g across all tanks with no significant differences in weight between replicates. Water temperature was maintained at 17±1°C and salinity at 29±1 ‰.

Dragon Research Ltd. fish meal based feed was used, with an average pellet diameter of 3.5 mm; the ingredients and composition are listed in Table 1.

Table. 1. Formulation and proximate composition of Dragon Research Ltd. fish meal based feed used in Experiment 1, 3.5 mm diameter pellets.

Feed ingredients %	
Fish meal	50.0
Wheat feed	41.0
Dried seaweed	2.0
Vitamin premix	2.0
Vegetable oil	5.0
Proximate composition	
Dry matter %	94.07
Protein %	40.63
Ash %	12.64
Energy kJ g^{-1}	18.00
Protein:energy mg kJ^{-1}	22.22

Four different feed rations were given daily based on the original size of the worms. In order to estimate the feeding capacity of *N. virens* a preliminary study in which worms were fed to satiation was performed (data not shown). Once a maximum ration was calculated for worms with an average weight of 4.5 g, the subsequent rations were deduced for use in the main experiment. Reduced rations of ¾ (high), ½ (medium) and ¼ (low) of maximum feed ration were tested, together with an unfed regime, as summarised below:

3 x 10 worms – unfed = 0 g ind^{-1} day^{-1}
2 x 10 worms – low = 0.025 g ind^{-1} d^{-1} (0.6 % worm body weight)
2 x 10 worms – medium = 0.05 g ind^{-1} d^{-1} (1.1 % worm body weight)
2 x 10 worms – high = 0.075 g ind^{-1} d^{-1} (1.7 % worm body weight)
3 x 10 worms – maximum = 0.1 g ind^{-1} d^{-1} (2.2 % worm body weight)

Three replicates were used for the unfed and maximum feed rations as a large amount of data points would be required for future studies involving bioenergetic modelling of the feed requirements of *N. virens.* The experiment duration was 50 days; before the worms were bagged and frozen, a sample of coelom was extracted using 1 ml syringe and 23G x 1" needle. The coelom sample was observed under a x40 objective on a Vickers microscope. Oocyte presence and diameter was recorded in order to establish maturity stage as this has been shown to affect feed intake (mature worms with large oocytes will cease feeding and focus energy on gonad growth).

Regression analysis and one-way ANOVA was performed for data analysis, as presented in General Materials and Methods.

3.2.2 Experiment 2: Effects of Increasing Feed Rations – Small Sized Worms

This experiment followed the same general methodology as the previous experiment but differed in the following ways. Firstly, worm size was smaller with mean individual size of 2.5 g. Secondly, a smaller pellet was used with a diameter of 1 mm. A preliminary study of *N. virens* feeding on large and small pellets was performed

(data not shown). The small size was favoured over larger pellets; worms consumed more feed and the small size also enabled more pellets to be distributed around the tub more evenly and hence more accessible to all worms. The feed was manufactured at Dragon Research Ltd.; ingredients and composition are presented in Table 2.

Table. 2. Formulation and proximate composition of Dragon Research Ltd. fish meal based feed used in Experiment 2, 1 mm diameter pellets.

Feed ingredients %	
Fish meal 70	60.0
Wheat starch	30.5
Rapeseed oil	4.0
DCP	1.0
Vitamin mix	0.5
Zeofeed (silica based filler)	3.5
Alginate (binder)	0.5
Proximate analysis	
Dry matter %	90.20
Protein %	39.86
Ash %	12.23
Energy kJ g^{-1}	17.87
Protein:energy mg kJ^{-1}	22.33

Communal tubs each containing 12 worms were used as replicate units for the fed groups. Individual pots were only used with unfed animals to prevent cannibalism (following Experiment 1, communal tubs had been found to achieve greater growth and survival rates and higher feed consumption). The experiment was designed as follows:

6 x 6 worms = Unfed (individual pots)
2 x 12 worms = 25 % of maximum ration
2 x 12 worms = 50 % of maximum ration
2 x 12 worms = 75 % of maximum ration
3 x 12 worms = 100 % of maximum ration

Since the feed was different, both physically and compositionally to the larger 3.5 mm feed used in Experiment 1, a preliminary study was undertaken with 2.5 g worms to calculate maximum feeding ration (maximum ration being deduced by amount of leftovers remaining after 24 h). Maximum ration was adjusted during this experiment as the worms consumed more feed; reduced rations were altered proportionately. Experiment duration was 39 days.

Regression analysis and one way ANOVA was performed for data analysis, as presented in General Materials and Methods.

3.2.3 Experiment 3: Starvation Effects on *N. virens*

The objective of this experiment was to assess whether *N. virens*, from which pelleted food was withheld, were able to obtain nutrition from organic material in the water or in the sediment. Two water supplies were used, one with standard seawater and another with recirculated sterilised water. For the sterilised treatments, a 150 litre sump stored water and a pump allowed circulation into the trough in which the tubs were placed. Sterile seawater was treated with UVC and ozone. Standard untreated seawater pumped directly from the sea was used in treatments involving normal seawater in the wet laboratory. Individual pots were again used to prevent cannibalism, as none of the worms were receiving feed.

4 different treatments were tested:

SSW + NSD - 3 x 6 worms - Sterile seawater and normal sand habitat (troughs)

SSW + SSD - 3 x 6 worms - Sterile seawater and sterile sand habitat (troughs)

NSW + NSD - 3 x 6 worms - Unsterilized seawater and normal sand habitat (wet laboratory)

NSW + SSD - 3 x 6 worms - Unsterilized seawater and sterile sand habitat (wet laboratory)

Sediment was rendered sterile by heating sieved sand in a muffle furnace at 550°C for 12 hours. Water temperature in both rooms was kept constant at 16°C±1. Experiment duration was 35 days and the mean individual worm wet weight was 3.91 g.

A two-way ANOVA was performed in this experiment as two variables were tested, sand and water quality.

3.3 Results

3.3.1 Experiment 1: Effects of Increasing Feed Rations – Medium Sized Worms

A summary of *N. virens* dietary performance and composition for Experiment 1 is presented in Tables 3, 4 and 5. Mean cumulative survival rates were high, between 80 and 93 %, with no significant differences among treatments. As the worms consumed increasing feed rations, they also consumed increasing protein and energy, up to 4.1 mg g^{-1} day^{-1} and 189.8 J g^{-1} day^{-1} respectively. There were no significant differences in FCR among treatments, values ranging from 0.98 to 1.46. Weight gain was proportional to the amount of feed given for all the feeding rations, SGR was highest for the high feed ration (0.88 % day^{-1}) but decreased for the maximum feed ration.

Mean oocyte size was 35.7 μm, with no significant size differences among dietary treatments (data not shown). There were no significant correlations between oocyte size and worm size or oocyte size and feed intake. Worms weighing 8 to 9 g did not contain significantly larger oocytes than smaller worms, implying that none of the animals used were mature. Spermatophores were not seen at all.

Table. 3. Performance of N. virens, mean starting size of 4.5 g, fed increasing rations of Dragon Research fish meal based pellet, from unfed treatments to maximum feed intake. Mean values ± SD.

	Unfed	Low	Medium	High	Maximum
Initial weight g	4.41 ± 0.31	4.60 ± 0.12	4.49 ± 0.16	4.51 ± 0.10	4.49 ± 0.42
Final weight g	3.94 ± 0.08	5.19 ± 0.16	6.04 ± 0.12	7.01 ± 0.45	6.42 ± 0.31
Weight gain mg ind^{-1} day^{-1}	-9.48 ± 4.80	11.91 ± 5.80	31.04 ± 0.73	49.99 ± 6.83	38.51 ± 6.98
Survival %	93.3 ± 11.55	90.0 ± 0.00	85.0 ± 7.07	80.0 ± 0.00	83.3 ± 5.77
Feed intake mg ind^{-1} day^{-1}	-	15.55 ± 1.91	30.54 ± 2.27	49.59 ± 0.50	54.76 ± 1.50
Feed intake % day^{-1}	-	0.32 ± 0.04	0.59 ± 0.03	0.88 ± 0.03	1.02 ± 0.04
FCR	-	1.44 ± 0.54	0.98 ± 0.10	1.00 ± 0.13	1.46 ± 0.30
SGR % day^{-1}	-0.22 ± 0.11	0.24 ± 0.12	0.59 ± 0.03	0.88 ± 0.08	0.72 ± 0.15

Regarding body composition, % ash content was higher in all treatments at the end of the experiment than the initial samples (1.8 %), but there were no significant differences in % ash content between unfed and fed treatments; end point values ranged from 2.18 to 2.49 % (Table 4). Protein levels also increased relative to initial samples, from 9.1 % to a maximum of 10.9 %. The highest protein % per wet weight was found with worms receiving the high feed ration, which also showed the highest body protein gain of 1.3 mg g^{-1} day^{-1}. There were no differences in the Protein Retention Efficiency (PRE) between treatments (Table 5).

Table. 4. Proximate composition per g live weight N. virens, mean starting size of 4.5 g, fed increasing rations of Dragon Research fish meal based pellet, from unfed treatments to maximum feed intake. Mean values ± SD.

	Initial	Unfed	Low	Medium	High	Maximum
Dry matter %	18.16	19.45 ± 0.26	19.37 ±1.33	21.36 ± 0.60	22.43 ± 0.96	20.62 ± 0.37
Ash %	1.81	2.49 ± 0.08	2.24 ± 0.05	2.23 ± 0.12	2.42 ± 0.19	2.18 ± 0.18
Protein %	9.12	10.18 ± 0.19	9.72 ± 0.10	10.38 ± 0.45	10.92 ± 0.15	10.01 ± 0.37
Energy J g^{-1}	4176	4264 ± 81.81	4229 ± 226	4964 ± 581	5164 ± 14	4808 ± 329

As can be seen in Table 4, the highest body energy (J g^{-1}) levels were found in the medium, high and maximum feed rations, ranging between 4808 and 5164 J g^{-1}. High feed ration resulted in the highest energy gain (62 J g^{-1} day^{-1}), followed by the medium (42.3 J g^{-1} day^{-1}) and maximum rations (45.1 J g^{-1} day^{-1}) (Table 5).

No differences in the Energy Retention Efficiency (ERE) were detected among treatments.

Table. 5. Energy and protein efficiencies of N. virens, mean starting size of 4.5 g, fed increasing rations of Dragon Research fish meal based pellets, from unfed treatments to maximum feed intake. Mean values ± SD.

	Unfed	Low	Medium	High	Maximum
Protein consumed mg g^{-1} day^{-1}	-	1.27 ± 0.15	2.34 ± 0.11	3.53 ± 0.12	4.09 ± 0.17
Protein gain mg g^{-1} day^{-1}	-0.07 ± 0.07	0.35 ± 0.13	0.84 ± 0.13	1.26 ± 0.12	0.92 ± 0.12
PRE %	-	25.59 ± 6.80	34.05 ± 7.03	33.92 ± 4.41	20.20 ± 4.37
Energy consumed J g^{-1} day^{-1}	-	61.27 ± 4.52	114.08 ± 9.14	172.28 ± 5.31	189.77 ± 6.79
Energy gain J g^{-1} day^{-1}	-10.07 ± 1.91	12.81 ± 9.63	42.32 ± 6.56	61.99 ± 2.63	45.06 ± 5.63
ERE %	-	20.39 ± 14.22	37.45 ± 8.75	36.03 ± 2.64	23.77 ± 3.17

3.3.2 Experiment 2: Effects of Increasing Feed Rations – Small Sized Worms

A summary of *N. virens* growth performance and body composition is presented in Tables 6, 7 and 8. Mean cumulative survival rates were high, between 83 and 100 %, with no statistically significant differences among treatments. As seen previously with Experiment 1, when feed increased, so did protein and energy intake, up to 13.2 mg g^{-1} day^{-1} and 562.4 J g^{-1} day^{-1} respectively. There were no statistically significant differences in FCR for the low, medium and high rations, which ranged between 0.72 and 0.98. However, the worms fed at maximum ration had a significantly higher FCR of 1.41 ($p<0.05$). Weight gain increased with increasing feed ration up to maximum feed intake where weight gain was decreased relative to the high feeding ration. This trend

was also reflected in the SGR, high feeding levels resulted in an SGR of 2.48 % day^{-1} while the maximum feeding level showed a lower SGR of 2.19 % day^{-1}.

Table. 6. Performance of N. virens, mean starting size of 2.5 g, fed increasing rations of Dragon Research fish meal based pellet, from unfed treatments to maximum feed intake. Mean values ± SD.

	Unfed	**Low**	**Medium**	**High**	**Maximum**
Initial weight g	2.48 ± 0.07	2.57 ± 0.22	2.58 ± 0.17	2.55 ± 0.04	2.43 ± 0.12
Final weight g	2.53 ± 0.05	4.31 ± 0.06	5.75 ± 0.56	6.71 ± 0.39	5.72 ± 0.48
Weight gain mg ind^{-1} day^{-1}	1.25 ± 2.22	44.63 ± 4.05	81.20 ± 10.18	106.57 ± 8.91	84.33 ± 14.35
Survival %	92.0 ± 8.33	92.0 ± 11.79	100.0 ± 0.00	83.0 ± 0.00	92.0 ± 0.00
Feed intake mg ind^{-1} day^{-1}	-	32.32 ± 3.23	61.05 ± 0.09	103.78 ± 1.82	117.25 ± 8.01
Feed intake % day^{-1}	-	0.97 ± 0.15	1.59 ± 0.13	2.51 ± 0.05	3.15 ± 0.18
FCR	-	0.72 ± 0.01	0.76 ± 0.10	0.98 ± 0.06	1.41 ± 0.16
SGR % day^{-1}	0.05 ± 0.09	1.33 ± 0.18	2.05 ± 0.09	2.48 ± 0.11	2.19 ± 0.31

The proximate composition of *N. virens* is presented in Table 7. % ash content and % dry matter were highest for the unfed animals (3.04 % and 16.98 % respectively), with no statistically significant differences observed among the other treatments. The highest % protein content per wet weight was found in the worms fed the medium (10.38 %) and high (10.92 %) feed rations.

Table. 7. Proximate composition per g live weight of N. virens, mean starting size of 2.5 g, fed increasing rations of Dragon Research fish meal based pellet, from unfed treatments to maximum feed intake. Mean values ± SD.

	Initial	**Unfed**	**Low**	**Medium**	**High**	**Maximum**
Dry matter %	18.10	16.98 ± 0.37	19.90 ± 0.76	21.95 ± 1.50	21.61 ± 1.15	21.39 ± 0.41
Ash %	2.68	3.04 ± 0.16	2.30 ± 0.08	2.28 ± 0.35	2.04 ± 0.31	1.99 ± 0.08
Protein %	9.12	9.86 ± 0.15	9.72 ± 0.10	10.38 ± 0.45	10.92 ± 0.15	10.22 ± 0.29
Energy J g^{-1}	3747	3240 ± 76	4483 ± 157	4969 ± 205	5016 ± 196	4943 ± 83

Table 8 presents the calculated energy and protein efficiencies of the 2.5 g worms. As consumed protein increased, so did body protein gains up to maximum protein levels consumed which showed a decrease in protein gain. Body protein levels decreased for starved worms, while the medium, high and maximum rations displayed protein gains ranging between 2.4 and 2.7 mg g^{-1} day^{-1}. PRE levels were high for the low, medium and high treatments, between 25.6 and 35.6 %. The maximum ration had a significantly lower PRE of 19.1 %.

The worms receiving medium, high and maximum feed rations contained body energy levels between 4943 and 5016 J g^{-1} and energy gains of 125.7 to 149.1 J g^{-1} day^{-1}. Higher energy consumed resulted in increased energy gains in the body apart

from the maximum intake of energy which showed a decrease in energy gain in the body. The lowest ERE was found for worms fed the maximum ration, with 23.4 %.

Table. 8. Energy and protein efficiencies N. virens, mean starting size of 2.5 g, fed increasing rations of Dragon Research fish meal based pellet, from unfed treatments to maximum feed intake. Mean values ± SD.

	Unfed	Low	Medium	High	Maximum
Protein consumed mg g^{-1} day^{-1}	-	4.09 ± 0.61	6.68 ± 0.55	10.54 ± 0.21	13.22 ± 0.74
Protein gain mg g^{-1} day^{-1}	-0.17 ± 0.09	1.41 ± 0.24	2.35 ± 0.39	2.70 ± 0.02	2.52 ± 0.30
PRE %	-	34.40 ± 0.80	35.55 ± 8.70	25.63 ± 0.30	19.08 ± 1.92
Energy consumed J g^{-1} day^{-1}	-	174.22 ± 25.95	284.41 ± 0.04	448.44 ± 9.10	562.43 ± 31.68
Energy gain J g^{-1} day^{-1}	-11.24 ± 3.77	74.99 ± 13.13	125.69 ± 23.46	149.09 ± 2.65	131.80 ± 16.41
ERE %	-	42.96 ± 1.13	44.52 ± 7.95	33.25 ± 0.08	23.40 ± 2.16

3.3.3 Experiment 3: Starvation Effects on *N. virens*

A summary of the performance of *N. virens* is presented in Tables 9, 10 and 11. Mean cumulative survival rates were high in all cases, ranging from 83 to 100 %, with no statistically significant differences among treatments. Statistically significant differences were detected for weight loss among the different treatments, with water quality having a significant effect ($p<0.05$). The worms undergoing the NSW + NSD treatment displayed the highest weight loss of -18.35 mg ind^{-1} day^{-1}. There was a trend for the NSW treatment to cause for a higher weight loss than SSW.

Table. 9. Performance of N. virens starved under different environmental conditions. SSW=Sterile seawater, NSW=Unsterilized seawater, NSD=Normal sand, SSD=Sterile sand. Mean values ± SD.

	SSW		NSW	
	NSD	**SSD**	**NSD**	**SSD**
Initial weight g	4.08 ± 0.27	4.11 ± 0.51	3.83 ± 0.17	3.65 ± 0.12
Final weight g	3.63 ± 0.23	3.64 ± 0.34	3.19 ± 0.17	3.17 ± 0.14
Weight gain mg ind^{-1} day^{-1}	-13.06 ± 1.53	-13.60 ± 5.24	-18.35 ± 1.70	-13.76 ± 1.95
Survival %	100.0 ± 0.0	83.0 ± 17.0	89.0 ± 10.0	89.0 ± 19.25
SGR % day^{-1}	-0.34 ± 0.03	-0.35 ± 0.10	-0.53 ± 0.06	-0.40 ± 0.06

Sediment organic content increased from initial values for NSD treatments (Fig. 2). Both water ($p<0.005$) and sand quality ($p<0.001$) had an effect on organic matter levels. The increase in organic content for the NSD treatment demonstrates that organic content must have accumulated in the sediment during the experiment.

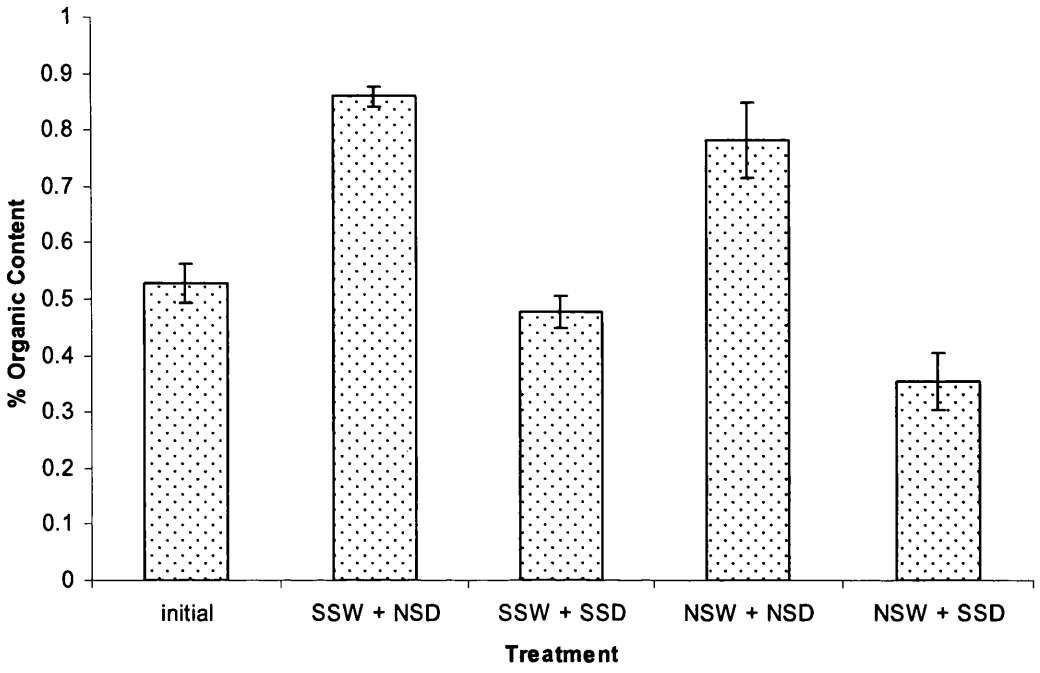

Fig. 2. Comparison of organic matter (% of dry weight) between initial and experimental sediment samples following the end of Experiment 3. SSW=Sterile seawater, NSW=Unsterilized seawater, NSD=Normal sand, SSD=Sterile sand. Mean values ± SD.

There were no significant differences in % dry weight (17.4 to 18.2 %) among the different treatments (Table 10). No differences could be observed among treatments regarding energy content of whole body, values ranged from 3932 to 4158 J g^{-1}.

Table. 10. Proximate composition per g live weight N. virens starved under different environmental conditions. SSW=Sterile seawater, NSW=Unsterilized seawater, NSD=Normal sand, SSD=Sterile sand. Mean values ± SD.

		SSW		NSW	
	Initial	**NSD**	**SSD**	**NSD**	**SSD**
Dry matter %	16.37	18.09 ± 0.21	17.40 ± 0.39	18.22 ± 0.48	18.17 ± 0.53
Ash %	1.43	1.83 ± 0.02	1.81 ± 0.10	1.97 ± 0.08	1.85 ± 0.02
Protein %	9.16	9.90 ± 0.12	9.74 ± 0.15	10.52 ± 0.09	10.19 ± 0.40
Energy J g^{-1}	3807	4117 ± 45	3932 ± 137	4100 ± 132	4158 ± 109

Energy loss was greatest (although not statistically significant) for NSW + NSD (-12.4 J g^{-1} day^{-1}) (Table 11). There were no statistically significant differences in protein gain or loss between the treatments, values ranging between -0.10 and -0.16 mg g^{-1} day^{-1}.

Table. 11. Loss of energy and protein in N. virens starved under different environmental conditions. SSW=Sterile seawater, NSW=Unsterilized seawater, NSD=Normal sand, SSD=Sterile sand. Mean values ± SD.

	SSW		NSW	
	NSD	**SSD**	**NSD**	**SSD**
Protein loss mg g^{-1} day^{-1}	-0.112 ± 0.06	-0.161 ± 0.07	-0.128 ± 0.03	-0.097 ± 0.06
Energy loss J g^{-1} day^{-1}	-4.54 ± 1.72	-9.81 ± 1.73	-12.38 ± 5.64	-6.08 ± 0.45

3.3.4 Combined Experiment Results

Small worms with an initial weight of 2.5 g were able to consume more feed relatively than larger worms, the maximum daily feed intake being 3.15 % compared to 1.02 % for the 4.5 g worms, and hence higher weight gain was achieved. The relationship of SGR to % feed intake is shown in Fig. 3. *N. virens* increased its growth with the amount of feed fed; however, for the highest rations, growth became level (4.5 g worms) or decreased slightly (2.5 g worms). The resulting curves can be described with the following equations:

(1) 4.5g worms: $y=-0.97x^2+1.98x-0.24$ $r^2=0.93$

(2) 2.5g worms: $y=-0.4x^2+1.89x-0.01$ $r^2=0.97$

where (y) is the SGR % and (x) is the Feed intake %

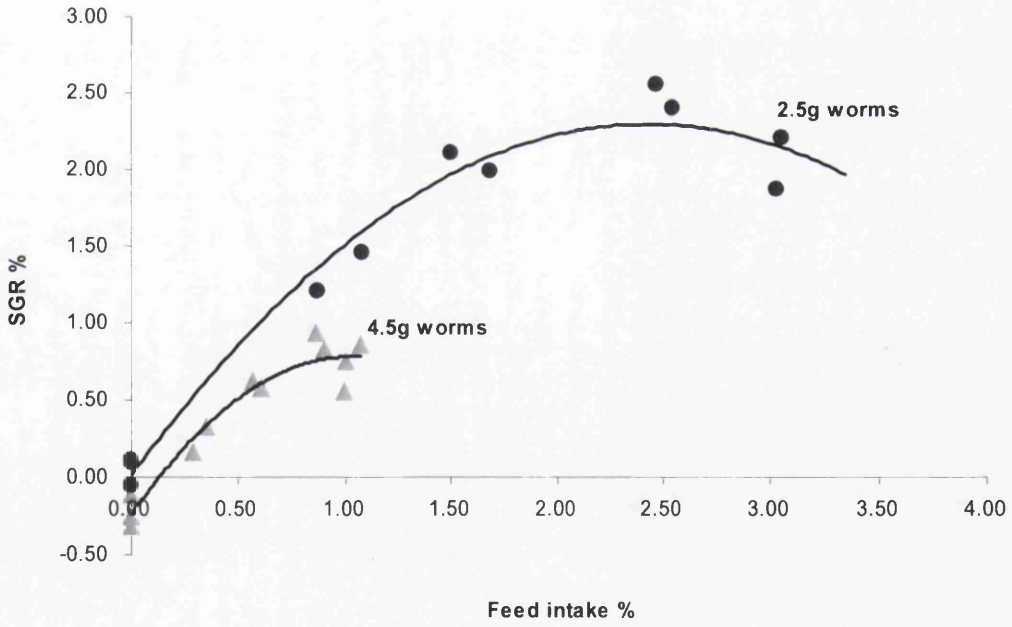

Fig. 3. SGR % day^{-1} of 4.5 g and 2.5 g N. virens (Experiments 1 and 2) in relation to feed intake %. Equations (1) and (2) described in the text.

The energy gain of both the 4.5 and 2.5 g worms receiving an increasing feeding ration is presented in Fig. 4, illustrating a curvilinear energy gain for increased calorific consumption. The 2.5 g worms were able to consume a higher amount of feed (and hence energy) than the 4.5 g worms, which led to higher energy gains per unit energy consumed. Energy gains increased with increasing energy consumed for both the 2.5 g and 4.5 g worms. The resulting curves can be described with the following equations:

(3) 4.5g worms: $y=-0.01x^2+0.6x-11.6$ $r^2=0.98$

(4) 2.5g worms: $y=-0.01x^2+0.64x-12.12$ $r^2=0.91$

where (y) is the Energy gain J g^{-1} day^{-1} and (x) is the Energy consumed J g^{-1} day^{-1}

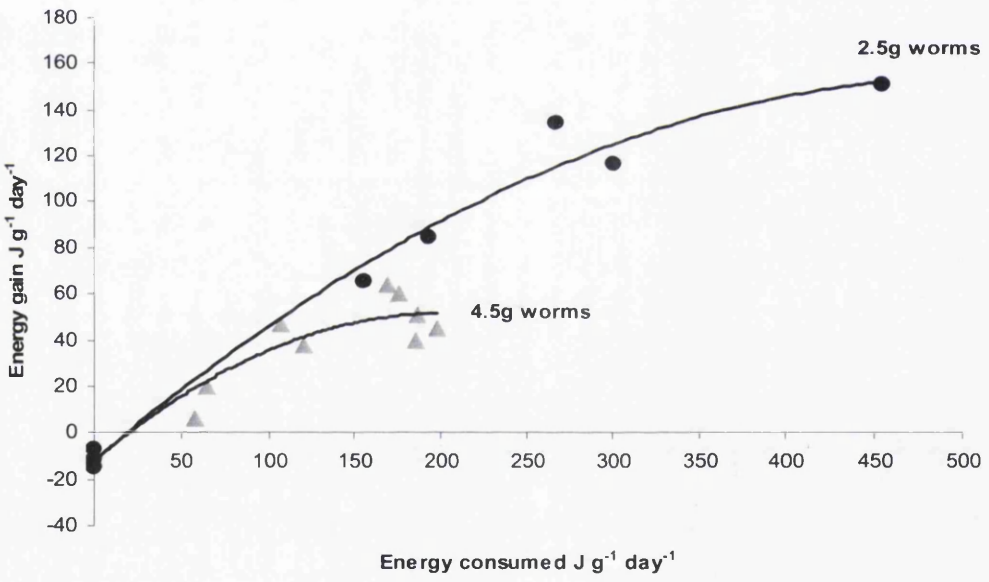

Fig. 4. Energy gained J g^{-1} day^{-1} in relation to energy consumed J g^{-1} day^{-1} for two different N. virens sizes: 4.5 g and 2.5 g, Experiments 1 and 2. Equations (3) and (4) described in the text.

Protein gain showed a similar trend relative to SGR for 2.5 and 4.5 g worms. When higher levels of protein were consumed for both weight classes of animals, protein gain either remained level or decreased slightly (Fig. 5). The relationship between protein consumed and protein gained for each weight class can be described using the following equations:

(5) 4.5g worms: $y=-0.07x^2+0.56x-0.11$ $r^2=0.90$

(6) 2.5g worms: $y=-0.02x^2+0.5x-0.18$ $r^2=0.96$

where (y) is the Protein gain mg g^{-1} day^{-1} and (x) is the Protein consumed mg g^{-1} day^{-1}

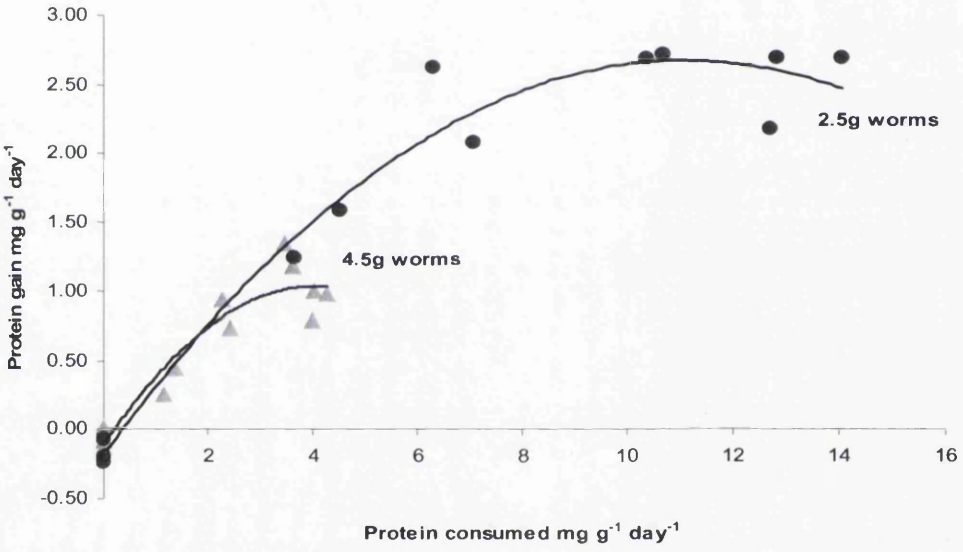

Fig. 5. N. virens protein gain mg g^{-1} day^{-1} in relation to amount of protein consumed mg g^{-1} day^{-1} for two different N. virens sizes: 4.5 g and 2.5 g (Experiments 1 and 2). Equations (5) and (6) described in the text.

No correlation was found between worm weights and the amount of energy loss during starvation, data combined for all 3 experiments (Fig. 6). Worms with a weight of 2.5 to 4.5 g lost approximately 2.5 to 15 J g^{-1} day^{-1} on average. *N. virens* under unfed conditions, in all three experiments, showed a dry matter content of 17 to 18.5 %. Thus % dry matter of unfed animals was always lower than of fed animals, which was between 20 and 23 %.

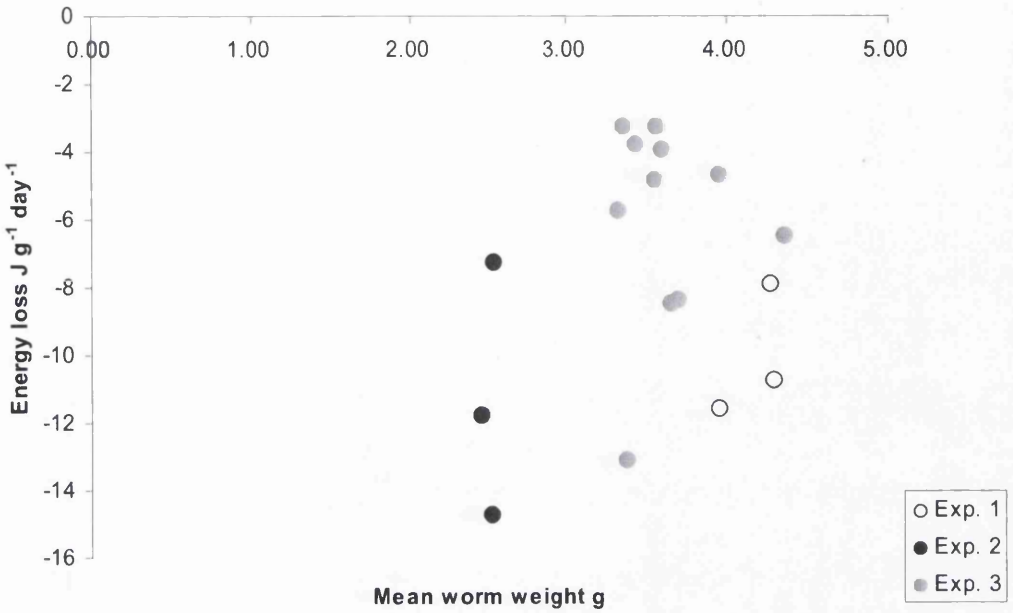

Fig. 6. Energy loss J g^{-1} day^{-1} in relation to weight (g) for 4.5 g (Exp. 1), 2.5 g (Exp. 2) N. virens and Experiment 3 worms, all subjected to unfed conditions.

3.4 Discussion

At low feed intake levels (Experiments 1 and 2, Tables 3, 5, 6 and 8), *N. virens* was not receiving sufficient nutrients as reflected in low growth rate, protein and energy gain. The priority use of ingested nutrients was likely to be metabolic requirements, with any excess nutrients being used for a small amount of growth and nutrient deposition in the tissues. Although not statistically significant in the case of the 4.5 g worms, ERE (20.39 %) and PRE (25.59 %) for worms fed the lower rations were also poorer (Table 5), which would be due to a fraction of the low energy intake being used for maintenance metabolism, at the expense of body growth.

Small, (2.5 g) *N. virens* displayed a higher daily feed intake (3.15 %) than larger, 4.5 g worms (1.02 %). This increase in feed intake may be due to the higher metabolic activity of smaller worms. This difference could also be due to the different characteristics of the feed pellets although both pellets contained similar protein and energy values. The pellet used for the larger worms was considerably larger (3.5 mm diameter) than that used for the smaller worms (1 mm diameter). As the ingestion capacity of the worms to consume both size pellets at both weight sizes was similar, the small sized pellet may be preferred for other reasons, perhaps involving feed size, manufacture or quality.

There was an increasing curvilinear shape to the nutrient gain - nutrient consumed relationship for both weight classes (Fig. 4 and 5). Regarding protein, around one quarter of the amount consumed was deposited as body protein; for example, 8 mg of protein consumed resulted in approximately 2 mg of protein gained (Fig. 5). For energy, the energy gain represented around one third of the amount consumed; for example, 300 J consumed translated into 100 J gained (Fig. 4). This model can serve as a tool in polychaete culture to predict nutrient gain from the amount of feed supplied.

Worms fed the maximum ration received the most feed and consequently the most protein and energy (Tables 5 and 8). However, there were no clear benefits in

feeding *N. virens* at apparent maximum feed intake over high feeding levels with regard to growth, protein or energy gain. Feeding the worms to satiation seemed to actually cause a decrease in the worm's ability to retain nutrients and growth rate; values were similar to the medium feed treatments. The highest FCR was found for the worms fed maximum rations, and FCR values closer to 1 for values beneath the maximum ration (Tables 3 and 6). A high FCR implies lower efficiency and a higher efficiency results in a low FCR. This is also a common feature found in many fish species such as rainbow trout, striped bass and channel catfish (Van Ham et al., 2003). The worms were consuming a high amount of feed and not converting the ingested material efficiently in the body, indicating that feeding to apparent satiation is excessive of the needs of *N. virens*. Furthermore, it is also more difficult to assess correct feed intake in *N. virens* compared to fish, which may have led to overfeeding of the experimental worms.

The highest protein and energy gain was found to be for worms, of both sizes, subjected to the high feeding rations, but not to satiation (Tables 5 and 8). This could be due to physiological inability of *N. virens* to utilize any more protein and energy and thus retain it in the body; the maximum feeding levels showed lower protein and energy gains. *N. diversicolor*, when cultured in a recirculating system, showed a similar trend to *N. virens*; additional energy intake did not result in extra growth for 600 mg worms fed less than 1 kJ day^{-1} (Bischoff, 2007). In shrimp, weight gain also decreased after reaching a maximum level, possible due to the unnecessary expenditure of energy in order to ingest feed; also, digestion efficiency may have been decreased due to faster passage rates associated with high rates of feed intake (Kureshy and Davis, 2002). The lower protein and energy retention at maximum feeding levels could perhaps be explained by the higher energy requirement for feed assimilation at higher feeding levels (Van Ham et al., 2003).

The decrease in weight gain at maximum protein levels may have also been due to inadequate non-protein energy necessary to metabolize excess absorbed amino acids, as seen in many fish species (Kim et al., 2005). Hence, it appears that for *N. virens*, the optimal feeding is at a high (0.88 and 2.51 % feed intake for 4.5 and 2.5 g worms

respectively), but not at maximum level, as feed efficiency, protein and energy gains tend to diminish.

The fact that oocyte size had no correlation with worm size or with feed consumption suggests that oocyte size was negligible at this stage; the worms were perhaps too young and there was no effect on the behaviour or physiology of the worms. Rapid oocyte growth occurs for worms that will breed in the following spring; oocytes double in diameter from 80 to 160 μm, when mature measure between 170 and 200 μm (Olive et al., 1998). With a mean oocyte size of 35.72 μm, the worms in the present study were not mature and hence are unlikely to have caused low feeding in certain individuals. Last and Olive (1999) estimate that oocytes found less than 80 μm were found in individuals that would be destined to spawn during the following or subsequent breeding season. Worms in the Year 1 class with a mean weight of 4.9 g were not fecund.

All worms undergoing unfed conditions showed high rates of survival (Tables 5, 8 and 9), with experiments ranging in length from 35 to 50 days. *N. virens* was able to withstand long periods without food, probably an adaption to estuarine conditions where food supply is unpredictable and/or due to the worms' low maintenance energy metabolism. In winter months, *N. virens* populations on the Thames estuary appear to have guts devoid of any obvious foodstuffs (Chapman and Taylor, 1968). Fasting can hence reach considerable duration before becoming critical in this species. Starved worms acquired more water then fed worms, which is shown in their lower dry weight levels relative to the fed worms (Tables 4 and 7). Water uptake may occur to maintain body weight and hence compensate for organic matter losses, such as decreases in carbohydrates and protein. The increase in water may also be due to the worms increasing their intake of water to extract nutrients.

The ash content (%) increased in starved worms, most likely due to the worm utilizing nutrients such as lipids and proteins which would be decreased during starvation conditions leading to an increase in non useable inorganic matter (Table 10).

Worm weight had no effect on the proportion of body energy lost during starvation experiments (Fig. 4). The sipunculid worm *Phascolosoma gouldii* when starved for three months had half the stored lipids utilized, protein content was decreased and 32.2 % of its dry weight was lost (Giese, 1966). Pocock et al., 1971, found that during starvation *N. virens* lost an increasing amount of weight; protein was broken down during maturation, whether the products of protein breakdown were merely eliminated or reused was not known.

In Experiment 3, worms may have demonstrated uptake of nutrients from the surrounding water and sediment for the NSW and NSD treatment, yet it would not be shown as energy or protein gain as they would be probably have been used for maintenance metabolism, respiration (Nielsen et al., 1995) and ventilation of water through the burrow, which has been found to be the predominant energy consuming activity of nereids (Miron et al., 1992). Christensen et al., 2000, found that *N. virens* spent more time as an active deposit feeder at the surface when algae was present than in a situation without algae. That study also found that the animals lost weight when phytoplankton was absent. Tita et al., 2000, observed that *N. virens* swallowed almost unselectively organic and inorganic fractions of the sediment; 75 to 98 % (depending on the time of year) of the gut was composed of sediment grains (Olivier et al., 1993).

The organic content of the unsterilized sediments increased during the experiment relative to the initial samples (Fig. 2). *N. virens* is unlikely to have taken advantage of this as a feed source as seen by the weight and nutrient losses. Bock and Mayer, 1999, found evidence that *N. virens* could ingest sediment to extract nutrients as the worms changed their digestive chemistry (enhanced presence of micelles when in sediment). Chapman and Taylor, 1968, showed that *N. virens* was able to uptake amino acids from the environment by diffusion as well as glucose and other small organic molecules.

Protein and energy loss for starved worms may have been lessened if the seawater and sediment had been more nutrient rich. This experiment demonstrates that besides the formulated feeds, no additional nutritive sources in the experimental

environment were available and that results gained from the non-fed worms can be accurately used for future maintenance and bioenergetic calculations (Chapter 5).

CHAPTER 4

Efficiency of Protein Utilisation of *N. virens*

4.1 Introduction

Protein in aquaculture feeds is of key importance and is usually the most expensive component. It generally consists of fish meal produced mainly from wild caught, small, pelagic fish, fish solubles or other marine invertebrate meal. The availability of fish meal is variable; loss of wild fish stocks is leading to increased cost of these raw materials. A significant amount of aquaculture research (Kaushik et al., 1995, Naylor, 2000) has focused on both finding alternative sustainable protein sources and optimizing the amount of protein fed to farmed animals. Suitable protein levels in feeds are established in the hope of obtaining high growth and healthy development of the animals and also preventing waste. Excess protein from uneaten feed or excretory products leads to increased ammonia production which can lead to degradation in water quality (New, 1996). Decreased water quality can have detrimental effects on the farmed animals as well as causing environmental effects in the wild (such as sea cages and shrimp ponds).

Protein is used for the growth of soft tissue and muscle, achieved by the synthesis and retention of protein (Fraser and Rogers, 2007). Proteins are also used in all types of metabolic activity in marine organisms and participate in all cell processes. Any protein in excess of these fundamental requirements is used for the energetic needs of the animals. Energy sources in diets come mainly from lipids and carbohydrates which are comparatively cheaper components than protein. Therefore, when inadequate energy is provided in diets, protein will be used as an energy source and not for growth (Lee, 2005). Replacing dietary protein with lipids would reduce nitrogen excretion, resulting from protein catabolism from energy; this requires decreasing the digestible protein to digestible energy ratio (Santinha, 1999), but can lead to higher fat deposition which may in certain cases decrease market value in species such as seabream. Not only should the energy concentration in the diet be considered primarily but also the ratio of protein to energy in order to optimize the use of both components in the body.

The ratio of protein to energy has been determined for many fish species such as bass (*Lates calcarifer)*, tilapia (*Oreochromis niloticus*) and carp (*Labeo rohita*) (Catacutan and Coloso, 1995, Das et al., 1991, ElSayed and Teshima, 1992). White seabream *Diplodus sargus* showed no difference in growth when the fish were fed high or low protein levels (Ozorio et al., 2006). This species is an opportunistic feeder whose main diet consists of algae and sea urchin. There are few similar studies performed with marine invertebrates (Mai et al., 1995) with the exception of farmed shrimp. In shrimp, no differences in growth or survival were found when fed 20 to 40 % dietary protein (Martinez-Cordova et al., 2002, Hopkins et al., 1995). Horn (1995) noted that feeding the herbivorous fish *Cebidichthys violaceus* high levels of protein did not result in higher levels of growth. Carnivorous fish such as salmon and bass require 40-55 % protein in the feed, omnivores and herbivores require less.

N. virens at Dragon Research Ltd. polychaete farm, at the time of study, were being fed a fish meal based diet containing 35-41 % protein. There were no known estimations of the requirements for protein of *N. virens* and this study aimed to establish an adequate level at which the worms can utilize protein for growth and metabolic regulation while preventing any protein waste that would degrade the environment. The objectives of this study were to look at how different levels of protein and subsequent protein to energy ratios in the diet affect the growth, development and ultimately the protein content in the body. This chapter also looked at the interaction between protein content of the feed and feeding level, as total protein consumption is the product of dietary content and actual feed intake. The performance and protein gain of *N. virens* when fed diets containing low protein levels at a high feeding ration was evaluated against worms receiving high protein diets at low feeding levels.

4.2 Materials and Methods

4.2.1 Experiment 4: Effects of Dietary Protein Level

Five protein levels were tested, ranging from 19 % to 45 % protein inclusion. Three replicate communal tubs of 12 worms each were assigned per diet, with a total of 15 tubs. Diets were formulated according to specification using the software Winfeed 2.8 and manufactured at Dragon Research Ltd. as outlined in Table 12.

Table. 12. Formulation and proximate composition of Dragon Research Ltd. fish meal based feed used in Experiment 4 with protein levels ranging from 19 to 45 %, 1 mm diameter pellets.

Feed composition %	**19% protein**	**23% protein**	**31% protein**	**40% protein**	**45% protein**
Fish meal 70	21.5	34.0	48.0	60.0	73.0
Wheat starch	66.0	54.5	40.5	30.5	18.0
Rapeseed oil	8.0	7.5	6.0	4.0	3.0
DCP	3.5	3.0	2.0	1.0	0
Vitamin mix	0.5	0.5	0.5	0.5	0.5
Zeofeed (silica based filler)	0	0	2.5	3.5	5
Alginate (binder)	0.5	0.5	0.5	0.5	0.5
Proximate composition					
Dry matter %	90.55	93.94	90.73	90.20	85.35
Ash %	7.97	7.74	9.94	12.23	12.82
Protein %	19.20	23.20	30.92	39.86	45.13
Energy kJ g^{-1}	17.82	18.42	18.25	17.87	17.49
Protein:energy mg kJ^{-1}	10.77	12.60	16.94	22.31	25.80

Feed pellet size was approximately 1 mm with an average weight of 2.9 mg. All feed was pre-weighed and given to the worms daily based on satiation levels (initially 1.5 % of body weight). Once the feed was distributed into the tub, the remainder was weighed to estimate the amount of feed eaten. Leftovers were counted and siphoned

out. Feed levels were adjusted depending on the remains in each tub in order to reach a satiation level. The experiment duration was 50 days.

A one-way ANOVA and regression analysis was performed for data analysis, as presented in General Materials and Methods.

4.2.2 Experiment 5: Combined Effects of Dietary Protein Levels and Feed Allowance

Two diets, the 19 % and 45 % dietary protein feeds formulated for the previous experiment were used here. Both diets were fed at high, medium and low rations as well as treatments without feed, with the following layout in communal tubs:

3 x 15 worms – 19 % protein level – high feeding ration
2 x 15 worms – 19 % protein level – medium feeding ration (½ high ration)
2 x 15 worms – 19 % protein level – low feeding ration (¼ high ration)

3 x 15 worms – 45 % protein level – high feeding ration
2 x 15 worms – 45 % protein level – medium feeding ration (½ high ration)
2 x 15 worms – 45 % protein level – low feeding ration (¼ high ration)

3 x 15 worms – Unfed treatment

High feeding ration was altered according to the amount of pellets leftover after 24 hours. If the worms consumed all the feed, high ration was increased and medium and low rations altered proportionately.

As there were two variables being investigated, dietary protein level and feed ration, a two-way ANOVA was used for statistical analysis.

4.3 Results

4.3.1 Experiment 4: Effects of Dietary Protein Level

N. virens survival, feeding and growth data are summarised in Table 13. Mean cumulative survival rates were high throughout the experiment, between 89 and 97 %, with no statistically significant differences among treatments. The feed intake of *N. virens* was similar across the feeds when fed to apparent satiation. The average amount of feed consumed was 169 mg ind^{-1} day^{-1} or 2.58 % of the worm's body weight. The FCR reached a low value of 1.11 for the high protein feed but FCR increased as feed protein levels decreased (as high as 1.95 for 19 % protein feed).

SGR increased as dietary protein increased (Fig. 7). The worms grown on the feed with the highest protein content had the highest SGR of 2.00 % day^{-1}. Feeds containing between 19 and 31 % protein showed no differences in growth rates (SGR between 1.46 and 1.52 % day^{-1}).

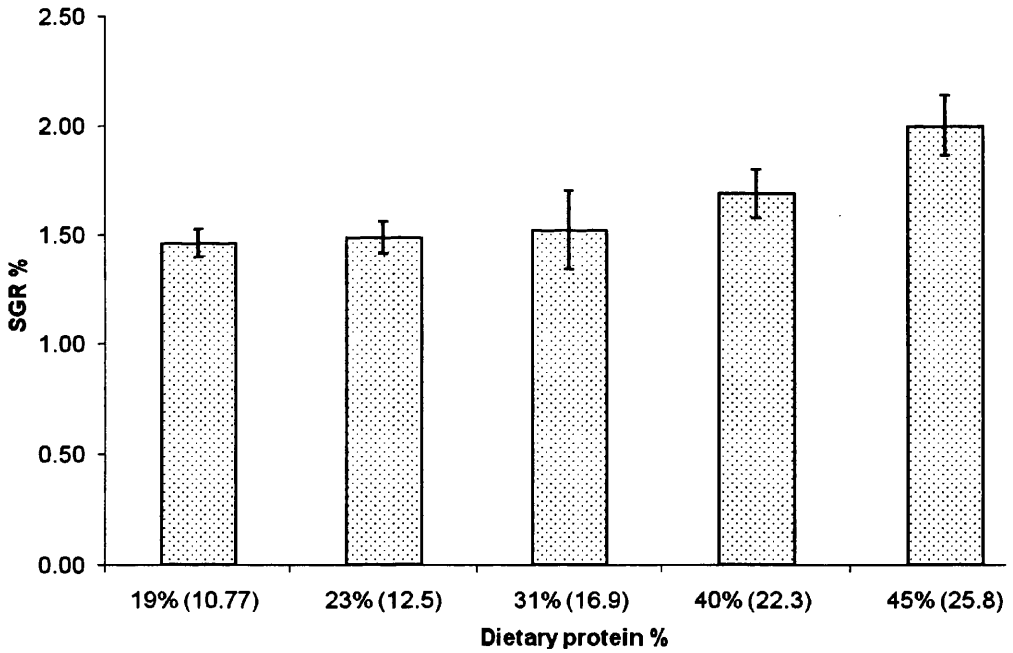

Fig. 7. SGR % day^{-1} of N. virens fed increasing dietary protein levels ($p<0.05$). Numbers in ( ) indicate the protein:energy mg kJ^{-1} of the different feeds. Mean values ± SD.

Table. 13. Performance of N. virens fed increasing dietary protein inclusion levels ranging from 19 to 45 %. Mean values ± SD.

	Feed protein level				
	19 %	**23 %**	**31 %**	**40 %**	**45 %**
Initial weight g	4.32 ± 0.09	4.29 ± 0.12	4.52 ± 0.23	4.45 ± 0.05	4.35 ± 0.12
Final weight g	8.97 ± 0.27	9.02 ± 0.29	9.68 ± 0.48	10.36 ± 0.48	11.83 ± 0.48
Weight gain mg ind^{-1} day^{-1}	93.16 ± 5.21	94.68 ± 5.61	103.25 ± 13.11	118.15 ± 10.44	149.53 ± 12.02
Survival %	91.67 ± 14.43	97.22 ± 4.81	88.89 ± 12.73	94.44 ± 4.81	97.22 ± 4.81
Feed intake mg ind^{-1} day^{-1}	180.42 ± 22.31	169.69 ± 15.68	163.68 ± 15.60	167.80 ± 4.61	165.02 ± 4.41
Feed intake % day^{-1}	2.91 ± 0.42	2.73 ± 0.20	2.48 ± 0.27	2.47 ± 0.02	2.30 ± 0.06
FCR	1.95 ± 0.33	1.79 ± 0.08	1.59 ± 0.15	1.43 ± 0.09	1.11 ± 0.11
SGR % day^{-1}	1.46 ± 0.06	1.49 ± 0.07	1.52 ± 0.18	1.69 ± 0.11	2.00 ± 0.14

There were no statistically significant differences in % dry matter (21.9 - 22.9 %) or % ash content (2.31 - 2.54 %) among treatments (Table 14). There were no statistically significant differences either in % protein content per wet weight among treatments; all had an average of 10.2 % protein wet weight, although there was a slight increase from the initial worm samples of 9.13 %.

Table. 14. Proximate composition per g live weight N. virens fed increasing dietary protein inclusion levels, ranging from 19 to 45 %. Mean values ± SD.

	Feed protein level					
	Initial	**19 %**	**23 %**	**31 %**	**40 %**	**45 %**
Dry matter %	16.93 ± 0.19	22.74 ± 0.39	22.85 ± 1.10	22.43 ± 0.29	22.51 ± 0.96	21.94 ± 0.62
Ash %	2.18	2.49 ± 0.49	2.39 ± 0.05	2.31 ± 0.12	2.48 ± 0.43	2.54 ± 0.55
Protein %	9.13	9.37 ± 0.94	10.18 ± 1.09	10.51 ± 0.12	10.48 ± 0.29	10.58 ± 0.40
Energy J g^{-1}	3650	5476 ± 323	5396 ± 103	5219 ± 112	5294 ± 449	5003 ± 161

The energy and protein retention efficiencies of *N. virens* are summarised in Table 15. Protein gain increased with increasing dietary protein levels up to 2.38 mg g^{-1} day^{-1} for the 45 % protein feed. There were however no differences in protein gain for feeds containing 19 to 31 % protein. PRE was highest for the worms fed the 19 to 31 % protein diets (Fig. 9).

The amount of energy consumed was slightly higher for worms fed low protein feeds, but there were no differences in the amount of energy gained across all protein treatments, values ranged from 102.93 to 120.77 J g^{-1} day^{-1}. ERE was high for worms fed the 45 % protein feed, reaching 30.05 %, compared to animals fed low protein diets, which showed no differences in ERE, which ranged between 21.10 and 25.67 % (Fig. 9).

Table. 15. Energy and protein efficiencies of N. virens fed increasing dietary protein inclusion levels, ranging from 19 to 45 %. Mean values ± SD.

	Feed protein level				
	19 %	**23 %**	**31 %**	**40 %**	**45 %**
Protein consumed mg g^{-1} day^{-1}	4.36 ± 0.63	6.54 ± 0.48	8.18 ± 0.90	10.38 ± 0.09	11.74 ± 0.31
Protein gain mg g^{-1} day^{-1}	1.44 ± 0.33	1.69 ± 0.27	1.83 ± 0.21	2.00 ± 0.21	2.38 ± 0.24
PRE %	33.58 ± 9.72	26.13 ± 5.74	22.45 ± 2.43	19.27 ± 1.85	20.32 ± 2.19
Energy consumed J g^{-1} day^{-1}	517.87 ± 75.03	501.99 ± 36.80	452.54 ± 49.92	441.65 ± 3.76	402.61 ± 10.65
Energy gain J g^{-1} day^{-1}	107.25 ± 8.13	106.17 ± 2.43	102.93 ± 7.62	113.37 ± 10.11	120.77 ± 10.26
ERE %	21.10 ± 4.24	21.22 ± 1.56	22.89 ± 2.41	25.67 ± 2.35	30.05 ± 3.30

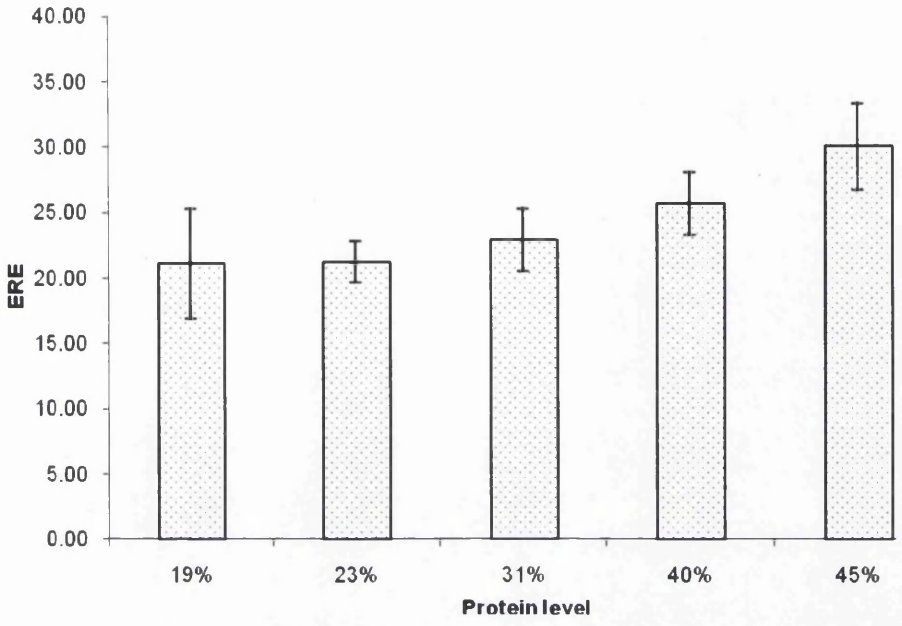

Fig. 8. Energy retention efficiency of N. virens fed different feed protein inclusion levels. Mean values ± SD.

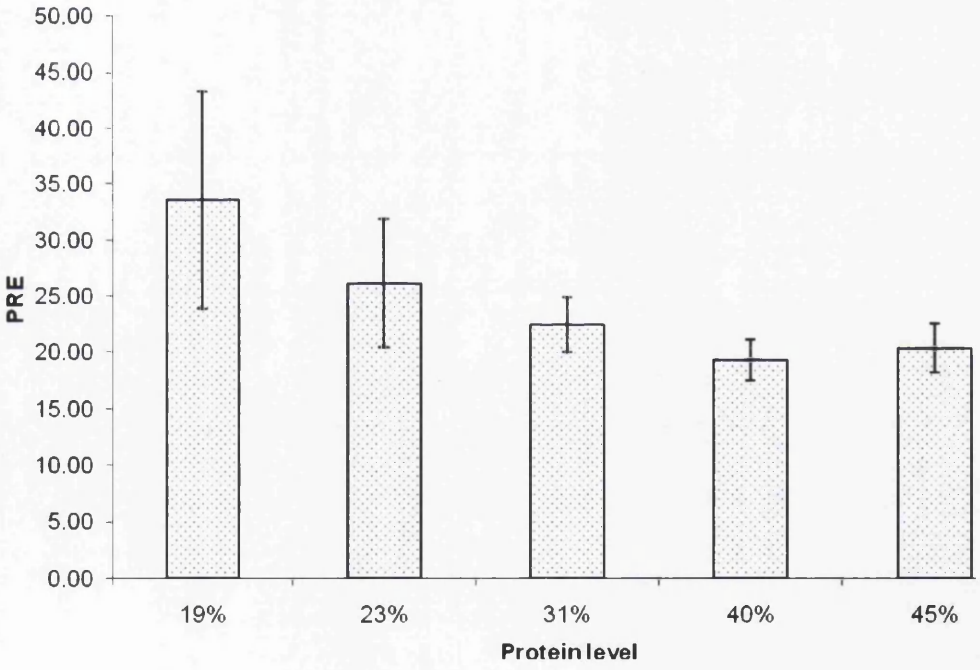

Fig. 9. Protein retention efficiency of N. virens fed different feed protein inclusion levels. Mean values ± SD.

4.3.2 Experiment 5: Combined Effects of Dietary Protein Levels and Feed Allowance

The performance of *N. virens* subjected to low and high dietary protein feed combined with increasing feed rations is presented in Table 16. Both the protein content and the feed ration had statistically significant effects on weight gain mg ind^{-1} day^{-1} ($p<0.001$ for both variables). The lowest weight gain of 57.63 mg ind^{-1} day^{-1} was found for animals fed low rations of the 19 % protein diet. A significant interaction occurred between protein content and ration level ($p<0.05$) for final weight results. The interaction arises due to the low and medium feed rations for the 19 % protein level having a smaller increment of weight gain (1.55 g) than for the same feed rations at the 45 % protein level (3.43 g). Highest weight gain was observed in the high 45 % protein ration, with 222.7 mg ind^{-1} day^{-1}.

Worms fed the medium ration 19 % protein consumed 1.58 mg protein g^{-1} day^{-1} and had similar weight gain to worms subjected to the low ration 45 % protein where they consumed 1.87 mg protein g^{-1} day^{-1} (weight gains of 103.6 and 95.6 mg wet weight ind^{-1} day^{-1} respectively). Those fed high ration 19 % protein showed similar growth to the medium ration 45 % protein treatment (weight gains of 152.5 and 170.8 mg wet weight ind^{-1} day^{-1} respectively).

Mean cumulative survival rates were high across all treatments, with rates ranging between 83.3 and 97.2 % and were unaffected by either protein content or feed ration. Both protein content ($p<0.001$) and feed ration ($p<0.05$) had a statistically significant effect on FCR. The highest FCR was found for the worms fed the high ration 19 % protein, at 1.35.

Table. 16. Performance of N. virens fed with 19 % and 45 % protein inclusion feeds at increasing feeding rations, from unfed treatments to high feeding levels. Mean values ± SD.

		19 % protein			45 % protein		
	Unfed	**Low**	**Med.**	**High**	**Low**	**Med.**	**High**
Initial weight g	4.10 ± 0.26	4.47 ± 0.27	4.04 ± 0.07	4.14 ± 0.06	4.07 ± 0.22	4.26 ± 0.02	4.13 ±0.40
Final weight g	3.65 ± 0.25	6.95 ± 0.22	8.50 ± 1.04	10.70 ± 0.35	8.17 ± 0.36	11.60 ± 0.06	13.70 ± 0.47
Weight gain mg ind^{-1} day^{-1}	-10.38 ± 4.55	57.63 ± 1.08	103.64 ± 25.95	152.52 ± 7.84	95.55 ± 3.39	170.82 ± 1.88	222.68 ± 19.15
Survival %	97.22 ± 4.81	95.83 ± 5.89	95.83 ± 5.89	91.67 ± 8.33	95.83 ± 5.89	83.33 ± 23.57	97.22 ± 4.81
Feed intake mg ind^{-1} day^{-1}	-	49.62 ± 3.05	99.38 ± 6.11	205.35 ± 17.03	49.62 ± 3.05	118.82 ± 33.61	190.63 ± 11.34
Feed intake % day^{-1}	-	0.89 ± 0.10	1.70 ± 0.19	3.08 ± 0.20	0.86 ± 0.01	1.69 ± 0.48	2.54 ± 0.14
FCR	-	0.86 ± 0.04	1.00 ± 0.31	1.35 ± 0.09	0.52 ± 0.01	0.70 ± 0.20	0.86 ± 0.07
SGR % day^{-1}	-0.27 ± 0.12	1.03 ± 0.07	1.72 ± 0.33	2.21 ± 0.07	1.62 ± 0.02	2.33 ± 0.02	2.80 ± 0.30

The proximate composition of *N. virens* is presented in Table 17. The interaction between protein content and feed ration was the only statistically significant effect on % ash content ($p<0.05$). Percent ash content was higher for unfed animals, with 2.10 %. Percent dry matter was not affected by either dietary protein content or feed ration. % wet weight protein content was significantly affected by the protein content of the diet ($p<0.15$) but nor by ration level nor the interaction between the two variables. Energy

content of the animals was not statistically significantly different among treatments and ranged between 4556 and 4980 J g^{-1} live body mass.

Table. 17. Proximate composition per g live weight N. virens fed with 19 % and 45 % protein inclusion feeds at increasing feeding rations, from unfed treatments to high feeding levels. Mean values ± SD.

			19 % protein			45 % protein		
	Initial	**Unfed**	**Low**	**Med.**	**High**	**Low**	**Med.**	**High**
Dry matter %	16.71	17.02 ± 0.40	20.28 ± 0.47	19.99 ± 2.17	19.13 ± 0.16	19.83 ± 1.32	19.61 ± 0.60	21.04 ± 0.22
Ash %	1.72	2.10 ± 0.04	1.88 ± 0.02	2.25 ± 0.39	1.70 ± 0.07	1.90 ± 0.01	1.53 ± 0.21	1.87 ± 0.19
Protein %	10.01	10.25 ± 0.44	10.12 ± 0.07	9.52 ± 1.31	9.31 ± 0.16	10.65 ± 0.47	10.41 ± 0.48	10.70 ± 0.09
Energy J g^{-1}	3649	3601 ± 119	4625 ± 206	4675 ± 639	4556 ± 103	4561 ± 373	4665 ± 15	4980 ± 147

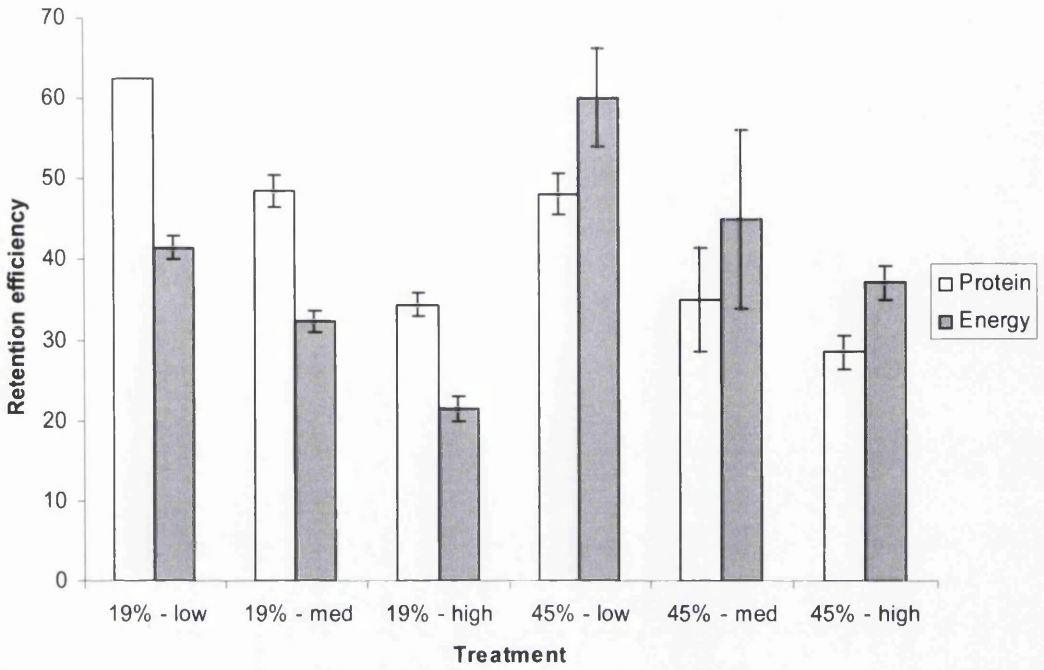

Fig. 10. Protein and energy retention efficiencies for N. virens fed with 19 % and 45 % protein feeds at increasing feeding levels (low, medium and high rations). Mean values ± SD.

Protein intake and nutrient efficiencies are summarised in Table 18. Protein gain and PRE were significantly affected by protein content ($p<0.001$) and feed ration ($p<0.001$). The highest protein gain was found to be for the medium and high ration 45 % protein, 2.59 and 3.27 mg g^{-1} worm day^{-1} respectively. The medium and high ration 19 % protein and the low ration 45 % protein showed similar gains of 1.58 - 2.03 mg g^{-1} day^{-1}. The low ration 19 % protein had the lowest gain with 1.07 mg g^{-1} day^{-1}. The highest PRE occurred for the low ration 19 % protein with 62.47 %. A lower PRE was found for the high ration 19 % protein (34.37 %), similarly to the medium (35.01 %) and high ration (28.51 %) 45 % protein.

Energy gain and ERE were significantly affected by protein level ($p<0.001$) and ration ($p<0.001$) (the amount of energy in the feed was formulated to be the same despite differences in protein level). Energy gains were slightly decreased for worms

receiving the 19 % protein diets relative to the 45 % protein diets, for each feeding level.

Differences arose for the ERE; worms fed the low ration 45 % protein diet had a higher ERE of 60.13 % than worms receiving the low ration 19 % protein with 41.50 %. A comparison between retention efficiencies in relation to the amount of nutrient fed is outlined in Fig. 10.

Table. 18. Energy and protein efficiencies for N. virens fed with 19 % and 45 % protein feeds at increasing feeding levels. Mean values ± SD.

		19% protein			45% protein		
	Unfed	**Low**	**Med**	**High**	**Low**	**Med**	**High**
Protein consumed mg g^{-1} day^{-1}	-	1.71 ± 0.18	3.27 ± 0.37	5.92 ± 0.39	3.88 ± 0.05	7.63 ± 2.16	11.46 ± 0.65
Protein gain mg g^{-1} day^{-1}	-0.22 ± 0.05	1.07 ± 0.09	1.58 ± 0.10	2.03 ± 0.11	1.87 ± 0.13	2.59 ± 0.16	3.27 ± 0.39
PRE %	-	62.47 ± 1.57	48.50 ± 2.42	34.37 ± 1.65	48.16 ± 2.76	35.01 ± 7.84	28.51 ± 2.37
Energy consumed J g^{-1} day^{-1}	-	159.11 ± 17.14	303.48 ± 34.59	549.53 ± 35.85	150.50 ± 1.87	295.59 ± 83.71	444.15 ± 25.32
Energy gain J g^{-1} day^{-1}	10.90 ± 3.38	66.13 ± 8.83	98.06 ± 6.29	117.46 ± 2.99	90.56 ± 11.35	127.63 ± 0.57	165.07 ± 17.68
ERE %	-	41.50 ± 1.08	32.41 ± 1.62	21.45 ± 1.86	60.13 ± 6.79	45.01 ± 12.94	37.11 ± 2.29

4.3.3 Combined Experiment Results

By combining the protein and energy data from Experiments 4 and 5, curvilinear trends concerning the relationship between nutrient consumed and nutrient gain were produced (Fig. 11 and 12). The resultant trends demonstrate that the protein gain increases before reaching a plateau as the amount of protein consumed increases. Regarding energetic values, energy gain increases with increased energy consumed, up to a feeding point of around 400-500 J g^{-1} day^{-1} when gain becomes reduced. A statistically significant correlation was found between protein or energy gain for *N. virens* in relation to the amount of nutrient consumed. The relationships are described with the following equations:

(7) Protein: $y=-0.018x^2+0.39x-0.003$ $r^2=0.89$

(8) Energy: $y=-0.001x^2+6.3x-6.4$ $r^2=0.81$

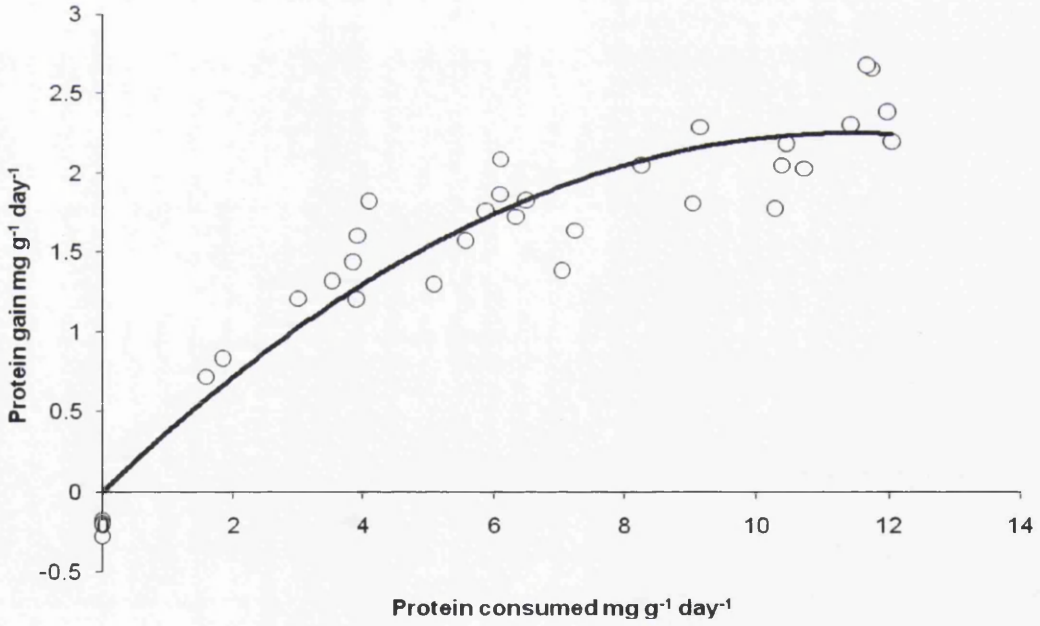

Fig. 11. Protein gain mg g^{-1} day^{-1} for N. virens in relation to the amount of protein consumed mg g^{-1} day^{-1} for Experiments 4 and 5. Equation (7) described in text.

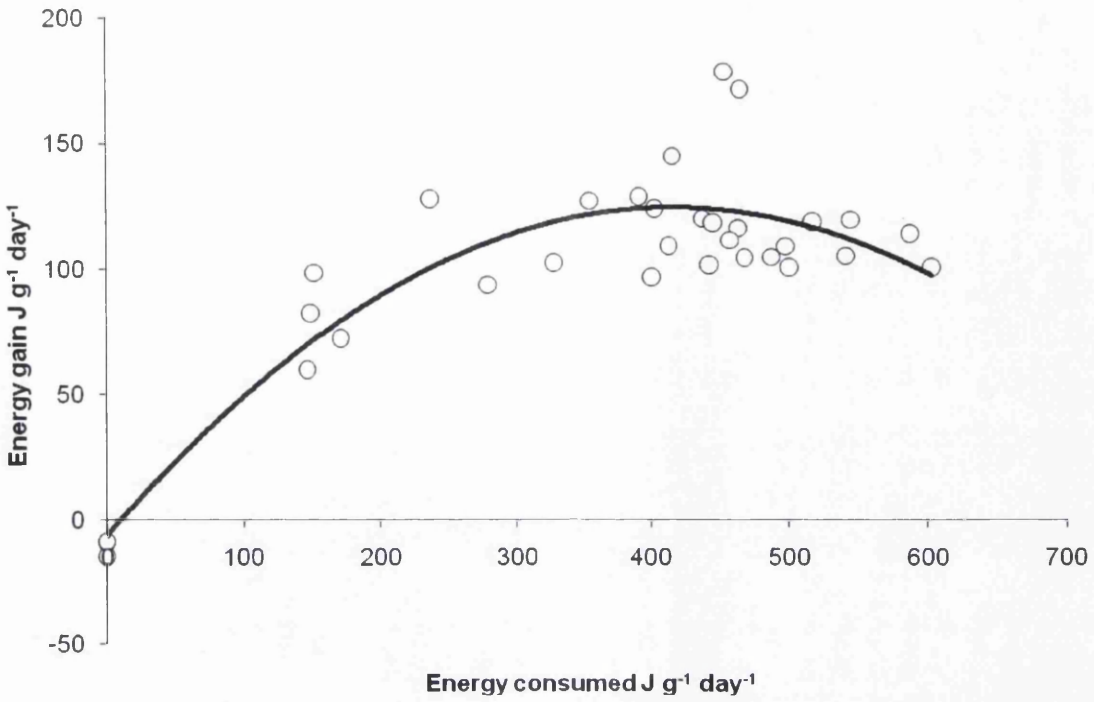

Fig. 12. Energy gain J g^{-1} day^{-1} for N. virens in relation to the amount of energy consumed J g^{-1} day^{-1} for Experiments 4 and 5. Equation (8) described in text.

4.4 Discussion

Growth was generally higher for worms in Experiment 5 than Experiment 4, despite similar starting sizes and diets (Tables 13 and 16). This difference between experiments could be due to the time of year at which the worms were sampled from the farm. Experiment 5 was conducted in June and Experiment 4 in August. Olive et al., 1997, noted that the life history reactions of semelparous organisms like *N. virens* are influenced by environmental variations. Breeding out of season is thought to lead to increased larval mortality and loss of fitness but the reasons for this are not well known. The differences in growth in these experiments may also be due to different breeding times or environmental temperatures in the polychaete farm raceways. Details of reproductive techniques were however not disclosed by Dragon Research Ltd. and the environmental conditions in the raceways leading up to the sampling of the worms on both occasions were not recorded.

Weight gain of *N. virens* in Experiment 4 increased with increasing protein ration up to 149.5 mg ind^{-1} day^{-1} for the 45 % protein ration, although there were no differences in growth for the three lowest protein inclusion feeds. Growth results in Experiment 5 demonstrated that the same results could be achieved by either feeding a low feed ration of higher protein diet or a medium feed ration of a low protein diet (Table 16); there were no statistically significant differences between the treatments. Similar weight gains also occurred for worms fed high rations of 19 % protein diet and for worms fed medium rations of 45 % protein diet. From these results, it appears that both protein and energy play important parts in the growth of *N. virens*. Olivier et al., (1996) suggested that nitrogen content of feed influences growth in the *Nereis* sp.; energetic food values represent a secondary factor in the control of growth of detritivorous species (Tenore, 1977, 1981, 1983). Few studies have been reported with polychaetes, although research carried out by Linton (2000) reported an increased growth of polychaete *Capitella* sp. with increased protein content of the sediment, *Capitella* sp. being deposit feeders.

In the case of the Pacific white shrimp *Litopenaeus vannamei*, weight gain was lower for shrimp fed a 10 % protein diet than shrimp fed higher protein diets (Aranyakananda and Lawrence, 1993); there were however no differences in weight gain for worms fed 15 % protein diets and above in the current study. The authors concluded that 15 % was the maximum dietary protein level with an optimal energy:protein of 28.57 kcal g^{-1} protein (or protein:energy of 8.36 mg kJ^{-1}). Other studies have found the optimal dietary protein level for the *L. vannamei* to be 30 %, although the energy:protein was maintained at 10 kcal g^{-1} protein (or protein:energy of 23.9 mg kJ^{-1}) (Cousin et al., 1991). According to Kureshy and Davis, 2002, greater weight gain was achieved by feeding a 32 % protein diet, lower weight gains occurred when fed a 48 % protein diet; this is thought to be due to the low energy to protein ratio of the 48 % protein diet which would cause the shrimp to utilize protein as a source of energy. High growth rates were also observed for the crayfish *Cherax quadricarinatus* when fed a high protein diet of 36 % (Cortes-Jacinto et al., 2005).

In Experiment 4, the high levels of energy consumed occurred for the low protein rations (Table 15). However, this high consumption was not translated into gain as there were no statistically significant differences among the entirety of the treatments in relation to the amount of energy gain. A high ERE was nevertheless observed for the two highest protein rations, 40 and 45 % (Fig. 8). This was also noted in the case of white seabream *Diplodus sargus*; fish fed high protein diets showed higher energy retention (Ozorio et al., 2006). It appears that the optimum protein:energy for *N. virens* would lie between 22.3 and 25.8 mg kJ^{-1}. At these values, the worms demonstrate high growth, survival as well high protein and energy gains in the body.

In Experiment 5, worms receiving high rations of the 19 % protein consumed the highest levels of energy but relatively low gain due to protein being a limiting factor (Table 18). The lowest ERE was found for the worms receiving a high ration of 19 % protein; a considerable amount of energy must be used to process large quantities of feed with low protein levels (Fig. 10). Conversely, highest ERE occurred for the low and medium ration 45 % protein; less energy was being utilised for food processing and

having high protein levels consumed for growth and metabolic activities resulted in more energy being available for deposition.

In Experiment 4, PRE decreased with increasing protein intake although weight gain increased. The highest PRE was found for the 19 % protein level ration; this treatment however displayed reduced weight gain (Table 15). The same trend for high protein gain and low PRE for high protein consumed was also seen in Experiment 5 (Table 18). Previous trials involving feeding rations and 2.5 g worms also showed an increase in PRE when fed low feeding levels (and hence lower protein levels) (See Chapter 3). These results demonstrate that a compromise needs to be found between growth and nutrient retention efficiency for *N. virens* when establishing feed regimes.

Protein gain was similar for the medium and the high rations of the 45 % protein feed. *N. virens* appears to show an inability to deposit any more protein when fed higher protein levels; the maximum gain was no more than 3.27 mg g^{-1} day^{-1}. As growth is defined by protein deposition, there is a limit to the growth potential of a certain species. These findings concur with results found in the case of many fish species such as tilapia (Kaushik et al., 1995), trout (Kim and Kaushik, 1992) and gilthead seabream (Vergara et al., 1996). White seabream *Diplodus sargus* fed low protein diets converted dietary protein into body protein more efficiently than fish fed the high protein diets (higher PRE) (Ozorio et al., 2006). In general, low protein intake in fish resulted in increased protein retention efficiency (Cho et al., 2001). High PRE found in worms receiving lower dietary protein resulted in less dietary protein being excreted or used as an energy substrate, these worms however showed reduced weight gain.

This outcome could be due to any excess of protein in the 40 % and 45 % protein rations becoming used as an energy source, as suggested in a study with gilthead seabream (Lupatsch et al., 2001). This would explain the higher energy content and ERE found for the high protein levels. Although it appears that the optimum protein requirement need reach no more than 40 % dietary protein in the feed in terms of protein and energy gains, the highest level of protein, 45 %, however did result in the highest growth of the animals, as well the lowest FCR of 1.11.

Feed ingestion rates were similar across all dietary protein treatments in Experiment 4 (Table 13). No compensatory response was observed for low dietary protein by increased ingestion, although energy levels were similar across all diets. There may be physiological reasons involving feed digestion times and gut fullness of the worm; the worms may only be able to process a given amount of feed in a time period. Similar results were found in shrimp *Litopenaeus vannamei*, where animals fed the 16 % protein diet showed a poor growth response as they could not compensate for an increased feed intake to meet daily nutrient requirements (Kureshy and Davis, 2002).

An energetic compensatory response has been shown for juvenile tilapia *Tilapia aurea*; the ingestion rate compensated for low energy density to maintain energy assimilation rate, but there was no compensatory response to low protein levels. Protein assimilation rate depends on the ratio of protein to energy (Bowen et al., 1995). Some fish species however may show an ability to consume higher amounts of feed in order to gain more protein; white seabream *Diplodus sargus* ingested more low protein feed, probably in order to try to meet their digestible protein and/or energy requirements (Ozorio et al., 2006). There were differences in ingestion rates for Experiment 5; the maximum ration for worms fed the 19 % protein diet (3.08 %) was higher than that of the maximum ration of worms fed the 45 % protein diet (2.54 %) (Table 16) As this effect did not occur in the previous experiment, this may be due to an experimental error when feeding or natural variation that occurs between farmed worm populations. The maximum feeding ration for the 19 % diet however resulted in the highest FCR of 1.35. This higher feed consumption of low protein diet did not result in efficient feed conversion. The lowest FCR occurred for the worms receiving a low feed ration of high protein, while all other treatments showed statistically significantly similar values, between 0.70 and 1.00 (Table 16).

The highest dietary protein inclusion level was 45 %; although untested, growth, protein gain and energy gain are likely to stagnate when fed levels above this, as seen in Chapter 3. A higher increase in dietary protein will cause the worms to presumably show a decreased performance. In a study with olive flounder *Paralichthys olivaceus*, Kim et al., 2005, found that when fed 65 % protein levels, weight gain was decreased.

The authors believed this may be due to inadequate non-protein energy being necessary to metabolise excess absorbed amino acids. An increase in protein accompanied by added carbohydrate resulted in increased weight gain for plaice (Cowey et al., 1975). In rainbow trout and Atlantic salmon, consumption of high energy feeds resulted in better growth, feed utilization and protein retention (Einen and Roem, 1997).

Worms fed the low protein feed need the entirety of their dietary protein and energy for metabolic processes and growth, hence they have a high PRE; the energy ingested would have been used to compensate for low protein intake. There is a substantial amount of evidence to show that protein is frequently the principal constraint to the growth, fecundity and survival of invertebrate and vertebrate primary consumers and that diet selection by these animals generally maximizes dietary protein:energy ratio (Bowen et al., 1995).

To conclude, the most efficient protein utilisation was obtained when feeding *N. virens* high rations of 45 % protein feed, with a protein:energy of 25.8 mg kJ^{-1}. This results in a suitable FCR of 0.86 to 1.11, an SGR of 2 - 2.80 % day^{-1}, as well as high protein and energy gains in the body. The only drawback of such a feeding regime appears however that some excess protein may be lost to the environment, as demonstrated by the low PRE. As the protein and energetic gains are not statistically significantly different to the worms fed the 40 % protein treatment, a dietary protein level between 40 and 45 % may perhaps be found to be more suitable.

CHAPTER 5

Bioenergetic Modelling of *N. virens* Nutritional Requirements

5.1 Introduction

The culture of *N. virens* is a relatively new and small scale industry. As a result, scientific research has been limited in the area of polychaete culture and has focused more on rearing techniques, including furthering knowledge of the life cycle and reproduction processes. Feed composition, manufacturing and delivery to the worms is currently at early stages of development and more research is needed to ensure that feed is not only sourced sustainably, but can also provide the necessary nutrients for *N. virens* growth and development, while remaining economical and non wasteful.

The polychaetes cultured in raceways by the industrial sponsor at the onset of this research were fed a fish meal based feed based loosely on the nutritional composition of fish and crustacean feeds used in aquaculture. Due to the sensitive nature of confidential feeding methods by the industrial sponsor, there was little information available on feed delivery to the worms. It is thought, however, that the worms were fed set amounts of feed with little or no consideration for worm size, protein or energy requirements. In order to improve the quality of production of *N. virens* as well as minimizing waste, suitable feed formulations need to be created based on the nutritional requirements of the worms for different weight classes. The use of such a customised diet will not only improve the efficiency of the worms to retain nutrients but also prevent unused feed components being excreted by the worms into the environment.

In this chapter, the series of data gathered in Chapters 3 and 4 served to create a bioenergetic model from which the protein and energy requirements for *N. virens* of different weight classes could be calculated. The average *N. virens* body energy and protein content was deduced as well as the potential weight gain and feed intake for a wide range of different sized worms. Coupled with the maintenance requirements for both protein and energy, these results served to create a mathematical model by which the nutritional requirements of *N. virens* at different sizes were established. The requirements were then used to produce a feeding table from which practical diets could be formulated.

5.2 Materials and Methods

A general description of potential weight gain for *N. virens* over the size range 1 - 14 g was performed by combining individual data points from high ration treatments across a range of experiments (n = 43 individuals) (Table 19). In these experiments, worms were fed to satiation and thus reflect the highest potential weight gain. The average worm weight between experiment start and finish was then taken using the geometric mean.

A wide-ranging nutritional proximate composition profile for *N. virens* was derived from a range of individual, end of experiment samples encompassing final weights (n = 41 individuals) (Table 20) and included samples taken at the start of the experiments. This data set also included worm samples of various sizes taken from the farm and analyzed in order to provide protein and energy values of whole body composition across a range of sizes.

An additional Experiment (no. 8) was performed solely to monitor the growth of *N. virens* in order to obtain growth and nutritional data starting from small sized worms of approximately 1 g. These worms were fed to satiation with a Dragon Research Ltd. 42 % fish meal feed and weighed fortnightly (Table 19). The first part of Experiment 8 used 2 tubs with 10 worms each, with an average wet weight of 1.95 and 2.72 g; the trial duration was 184 days. The second part of the experiment also used 2 replicate tubs with 10 worms each, but with an average worm wet weight of 0.99 and 1.03 g; the experiment duration was 103 days.

Only those worms that had received fish meal based diets were used in this analysis in order to eliminate any variation in nutritional composition due to feed sources.

This data set as well as the information derived from the starvation experiments (negative growth needed to establish maintenance values) allowed for the maintenance values for energy and protein to be deduced using the linear equation of the slope produced when plotting nutrient gain against nutrient consumed.

The efficiency of protein and energy utilization for both maintenance and growth was derived from Experiments 1, 2, 3 and 5, in which increasing levels of protein and energy had been fed to the worms.

A modelling approach based on Lupatsch and Kissil, 2005, was adapted to look at the requirements for growth and protein for *N. virens*.
The daily energy requirements of the worms encompassing both maintenance and growth were quantified using the equation:

Energy requirement (kJ ind^{-1} day^{-1}) = a x BW (g) + c x growth
Protein requirement (mg ind^{-1} day^{-1}) = a x BW (g) + c x growth

a = maintenance requirement
BW = body weight
c = cost of energy deposition (reciprocal of the slope in the nutrient consumed – nutrient gained relationship)
growth = amount of energy and protein gain respectively

The equation applied by Lupatsch and Kissil, 2005, contains an exponent *b*, a x BW $(g)^{b}$. *b* is the exponent of the metabolic body weight and converts absolute weight to metabolic weight correcting for the decrease in metabolic rate per unit of body weight as animals grow.
In the present trials with polychaetes this exponent was assumed to be b = 1 as worm weight range was not large enough to show any effect of worm weight on metabolic rate.

5.3 Results

5.3.1 Growth Potential

The weight gain g ind^{-1} day^{-1} of *N. virens* in relation to the worm weight (g) and feed intake can be seen in Fig. 13, data points used in Table 19. As worms increase in size, they consume higher amounts of feed. Feed intake also appears to be closely correlated with weight gain for worm sizes up to 3 g; for larger worms feed intake increases relative to the weight gain.

The potential weight gain of a worm of any size can be described with the equation $y = 0.015x^{1.106}$.

The feed intake of *N. virens* in relation to weight is described as $y = 0.013x^{1.325}$

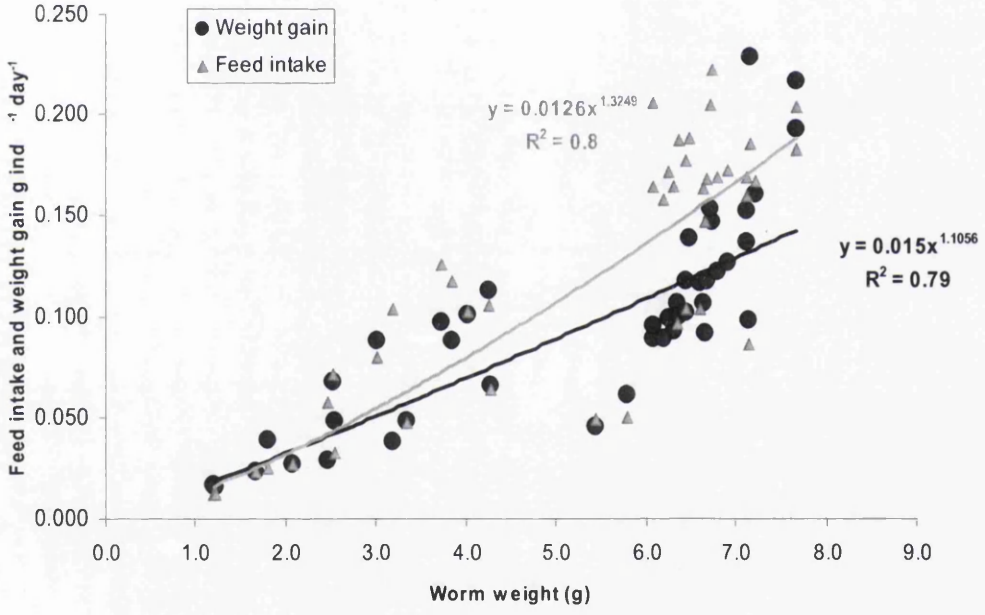

Fig. 13. Daily weight gain g ind^{-1} day^{-1} and feed intake g ind^{-1} day^{-1} in relation to increasing N. virens body weight (n = 43).

*Table. 19. Data points from Experiments 1, 2, 4, 5, 6 and 8 used for weight gain g day^{-1} and feed intake in g ind^{-1} day^{-1} (both wet weight). Mean weight based on the geometric mean of the final and initial weights between successive weighings. * Data points for Experiment 8 indicate the date at which replicate tubs were weighed.*

Experiment	Treatment	Mean weight g	Weight gain g ind^{-1} day^{-1}	Feed intake g ind^{-1} day^{-1}
Experiment 1	High feed	5.45	0.045	0.049
	High feed	5.80	0.061	0.050
Experiment 2	High feed	4.03	0.100	0.102
	High feed	4.25	0.113	0.105
	Max feed	3.84	0.088	0.117
	Max feed	3.74	0.097	0.125
Experiment 4	15% protein	6.08	0.088	0.206
	15% protein	6.32	0.093	0.164
	15% protein	6.27	0.099	0.171
	24% protein	6.20	0.089	0.157
	24% protein	6.08	0.095	0.164
	24% protein	6.38	0.100	0.187
	33% protein	6.68	0.091	0.147
	33% protein	6.46	0.102	0.177
	33% protein	6.69	0.117	0.168
	42% protein	6.65	0.106	0.163
	42% protein	6.81	0.121	0.168
	42% protein	6.91	0.127	0.172
	51% protein	7.24	0.160	0.167
	51% protein	7.14	0.152	0.160
	51% protein	7.13	0.136	0.168
Experiment 5	19% - high	6.75	0.146	0.222
	19% - high	6.50	0.138	0.188
	19% - high	6.72	0.153	0.205
	45% - high	7.67	0.217	0.204
	45% - high	7.68	0.193	0.183
	45% - high	7.18	0.229	0.186
Experiment 6	Control	6.36	0.106	0.096
	Fish meal	6.60	0.117	0.103
	Fish meal	6.45	0.117	0.103
Experiment 8 - Trial 1*	rep 1: 14/5/7	2.55	0.048	0.033
	rep 2: 14/5/7	2.08	0.027	0.027
	rep 1: 29/5/7	3.18	0.038	0.103
	rep 2: 29/5/7	2.48	0.029	0.057
	rep 1: 25/6/7	4.28	0.079	0.064
	rep 2: 25/6/7	3.35	0.058	0.047
	rep 1: 16/8/7	7.15	0.097	0.086
Experiment 8 - Trial 2*	rep 1: 7/2/8	1.20	0.017	0.012
	rep 2: 7/2/8	1.23	0.016	0.012
	rep 1: 25/3/8	1.81	0.039	0.025
	rep 2: 25/3/8	1.68	0.023	0.023
	rep 1: 14/4/8	3.01	0.088	0.079
	rep 2: 14/4/8	2.52	0.068	0.071

5.3.2 Body Composition

The protein, energy, ash and moisture composition of *N. virens* across the size range 1.6 to 14.0 g are presented in Fig. 14 and detailed in Table 20.

There were no significant changes with increasing worm size for any of these 4 variables. We can therefore assign an average value for each parameter:

Protein (mg g^{-1}): **101.41** (± 7.66)

Energy (J g^{-1}): **4822** (± 580)

Ash (mg g^{-1}): **22.77** (± 6.61)

Moisture (mg g^{-1}): **791.82** (± 22.44)

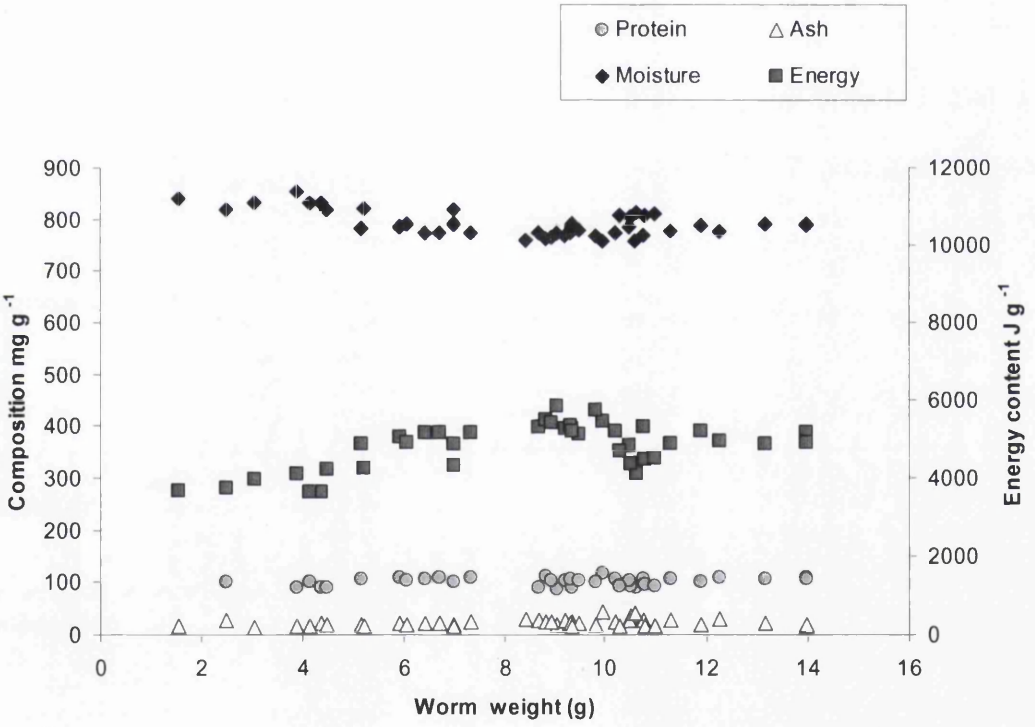

Fig. 14. Proximate body composition (mg g^{-1}) of N. virens, at various sizes fed to satiation (no. of sample sets = 41).

Table. 20. Data points from Experiments 1, 2, 4, 5, 6 as well as samples taken from Dragon Research Ltd. used for protein and energy composition for worms of different sizes.

Experiment	Treatment	Weight g	Protein content mg g^{-1}	Energy content J g^{-1}	Dry matter mg g^{-1}	Ash mg g^{-1}
Experiment 1	Initial	4.49	91.24	4219	181.60	18.05
	High feed	6.70	108.18	5173	224.30	22.90
	High feed	7.32	110.29	5155	224.40	25.54
Experiment 2	Initial	2.51	99.75	3747	181.00	26.82
	High feed	6.43	105.44	5155	224.20	20.35
	High feed	6.99	100.55	4878	207.97	22.55
	Max feed	5.92	110.31	5040	215.66	19.87
	Max feed	6.07	102.45	4907	209.20	20.73
	Max feed	5.18	107.36	4889	216.92	19.87
Experiment 4	Initial	9.13	91.34	3651	169.34	21.76
	15% protein	8.68	90.28	5320	224.34	27.05
	15% protein	9.04	86.60	5848	226.13	19.22
	15% protein	9.20	104.33	5261	231.68	28.37
	24% protein	8.81	110.87	5488	236.02	23.57
	24% protein	8.91	104.82	5415	233.58	24.57
	24% protein	9.35	89.75	5286	215.82	23.70
	33% protein	9.33	105.39	5337	226.60	22.76
	33% protein	9.49	103.72	5114	221.00	22.14
	33% protein	10.23	106.12	5207	225.40	24.54
	42% protein	9.83	101.90	5738	229.58	19.89
	42% protein	10.49	104.88	4841	213.95	26.40
	42% protein	10.77	107.72	5300	231.57	28.02
	51% protein	12.27	110.05	4932	223.01	29.49
	51% protein	11.89	102.05	5186	212.16	19.12
	51% protein	11.31	105.43	4890	223.02	27.70
Experiment 5	Initial	4.16	100.08	3649	167.10	17.16
	19% - high	10.80	94.70	4485	192.70	17.10
	19% - high	10.31	91.60	4674	191.70	17.70
	19% - high	10.99	93.00	4508	189.60	16.30
	45% - high	13.97	86.39	4120	170.30	13.54
	45% - high	13.16	84.94	3908	167.11	16.52
	45% - high	13.98	85.54	3926	167.59	14.77
Experiment 6	Initial	3.90	89.22	4092	146.00	15.74
	Control	9.97	117.08	5470	242.65	43.81
	Fish meal	10.63	89.52	4097	187.27	32.55
	Fish meal	10.52	93.61	4371	195.62	34.17
Farm Samples	-	1.55	92.16	3689	159.80	15.04
	-	3.05	93.21	3948	167.80	14.93
	-	5.21	100.71	4262	179.30	15.83
	-	6.98	101.54	4337	182.20	15.16
	-	9.34	104.40	5209	208.10	15.92

5.3.3 Maintenance Requirements

The maintenance requirements for protein are presented in Fig. 15 and energy in Fig. 16. Data points were taken from experiments in which graded feeding levels were tested and the relationship between feed intake and deposition assessed (Appendix Table 39). Data points beyond the linear range were omitted as these points represented a surplus of protein and energy consumed which would skew the values representing maintenance and retention efficiency values (See Chapter 3 Discussion).

The linear regression for the relationship between energy consumed and energy gained can be described by the equation $y = 0.42x - 7.56$. Maintenance requirement for energy can be deduced by substituting a value of 0 for the y value for zero energy balance. Therefore, for a worm to maintain its energy levels without any additional gains or losses it would need to consume 18 J g^{-1} day^{-1} energy.

Likewise, the linear regression for the relationship between protein consumed and protein gained can be described by $y = 0.36x - 0.07$, the maintenance requirement for protein can be deduced by substituting a value of 0 for the y value.
Therefore, for a worm to maintain a zero protein balance without any additional gains it would need to consume 0.19 mg g^{-1} day^{-1} protein.

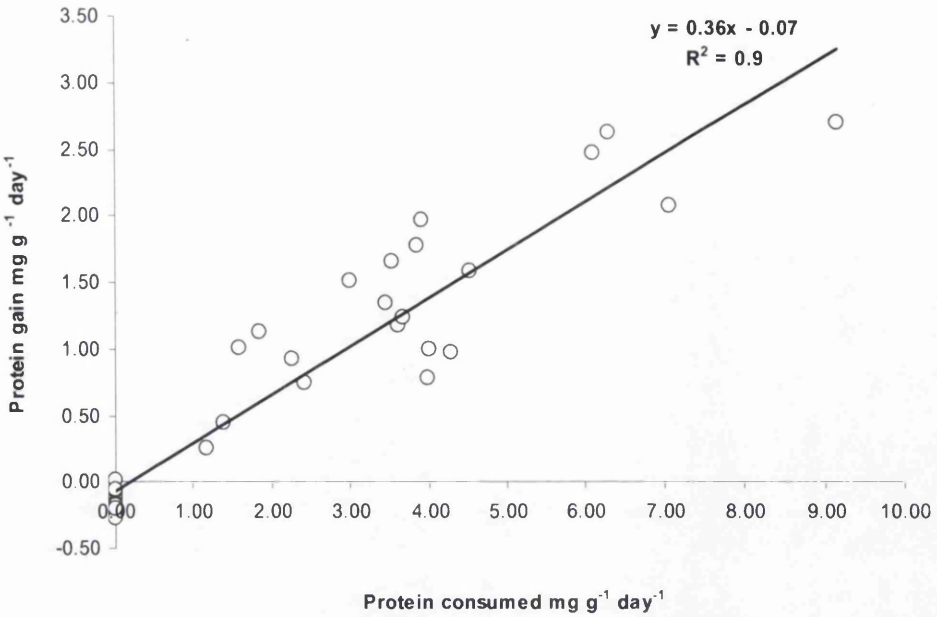

Fig. 15. N. virens protein gain mg g^{-1} day^{-1} in relation to amount of protein fed mg g^{-1} day^{-1} for different worm sizes. (Source data found in Appendix, Table 38), no. of sample sets = 42.

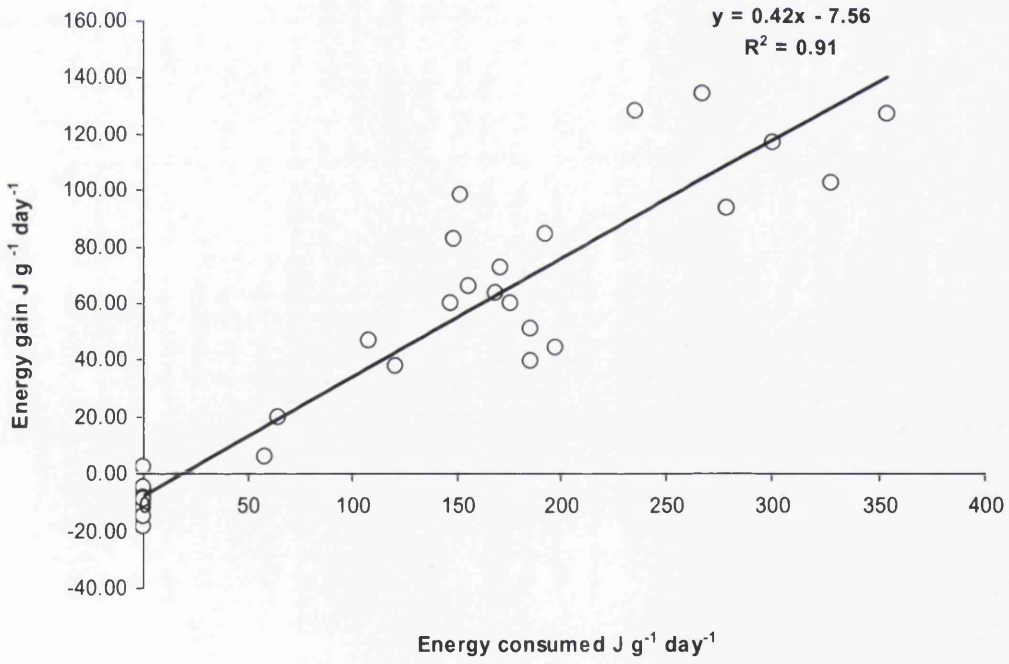

Fig. 16. N. virens energy gain J g^{-1} day^{-1} in relation to amount of energy fed J g^{-1} day^{-1} for different worm sizes (Source data found in Appendix, Table 38), no. of sample sets = 42.

5.3.4 Nutrient Requirements

a) Energy

As mentioned previously, average body energy content of *N. virens* was found to be **4822 J g^{-1}**.

Using both the maintenance requirements of the worms derived from the linear relationship $y = 0.42x - 7.56$ (Fig. 16) as well as the potential weight gain (and thus energy gain) of worms at different life stages (Fig. 13) the total energy requirements were calculated as:

Requirement (J ind^{-1} day^{-1}) = a x BW (g) + c x growth

c = cost of energy deposition
= 1/0.42
c = 2.38

a = maintenance requirement ($y = 0.42x - 7.56$)
a = 18 J g^{-1} day^{-1}

Maintenance for 5 g worm: a x BW (g) = 18 x 5
= 90 J day^{-1} 5 g worm

Using an example of a 5 g worm, the daily energy need in order to allow for optimal worm growth can be established.

growth – 5 g
c x growth

$y = 0.015 \times BW(g)^{1.106}$
$y = 0.015 \times 5^{1.106}$

$y = 0.089$ g day^{-1} 5 g worm

c x growth x average energy J in 5g worm = 2.38 x 0.089 x 4822

= 1021 J day^{-1} 5 g worm

Total requirement for a 5 g worm (maintenance + growth) = 1021 + 90

= 1111 J day^{-1} 5 g worm

This calculation can be applied to determine the energy requirements for growth for any size of *N virens* within the size range studied.

b) Protein

The average body protein content was found to be **101.41 mg g^{-1}**.

Using both the protein efficiency of the worms derived by the linear relationship $y = 0.36x - 0.07$ (Fig. 15) as well as the weight gain of worms at different life stages (Fig. 13) the total protein requirements for growth were calculated as:

Requirement (mg ind^{-1} day^{-1}) = a x BW (g) + c x growth

c = cost of energy deposition
= 1/0.36
= 2.78

a = maintenance requirement ($y = 0.36x - 0.07$)
a = 0.19 mg g^{-1} day^{-1}

Using again an example of a 5 g worm, the daily protein need in order to allow for optimal worm growth can be established.

c x growth x average protein mg in 5 g worm = 2.78 x 0.089 x 101.41

= 25.09

Maintenance for 5 g worm: a x BW (g) = 0.19 x 5

= 0.95 mg day^{-1} 5g worm

Requirement for a 5 g worm (maintenance + growth) = 25.09 + 0.95

= 26.04 mg day^{-1} 5g worm

This calculation can be applied to determine the protein requirements for growth for any size of *N. virens*.

Using the formula demonstrated above for 5 g worms, the nutritional requirements for worms of different sizes can be calculated, as seen in Table 21. By means of the formula $y = 0.013x^{1.325}$, deduced from the feed intake equation in Fig. 14, the daily voluntary feed intake can also be calculated.

Table. 21. Quantification of protein and energy requirements for N. virens, sizes 1-14 g.

Worm size g	Protein requirement mg ind^{-1} day^{-1}	Energy requirement J ind^{-1} day^{-1}	Weight gain mg ind^{-1} day^{-1}	Voluntary feed intake mg ind^{-1} day^{-1}
1	4.41	190	15	13
3	14.67	724	51	54
5	26.04	1111	89	106
7	37.98	1869	129	166
10	55.46	2727	191	266
12	67.12	3299	234	339
14	81.59	4005	278	416

5.4 Discussion

Growth data and feed intake information for *N. virens* was provided by an extensive database derived from a large number of preceding experiments (Table 19). All data points used were for worms fed to satiation and showing high growth. An accurate recording of the voluntary maximum feed intake was important as this would have an influence on the proper nutrient density of the feeds. Weight gain in *N. virens* appeared to be more dependent on feed intake than worm size (Fig. 13). Having established the maximum potential growth and the necessary energy and protein intake to meet this growth feed formulations can be devised in future that are tailored more closely to the worm's nutritional needs.

Growth potential for worms of increasing size displayed a linear trend; the larger the worm, the more weight gain it can achieve. There was no obvious cessation in growth for larger animals, it appeared to be continuous. Kay and Bradfield, 1973, estimated that *N. virens* can reach 15 g wet weight in two years. Further research could be carried out on larger animals, however, larger worms (>14 g) are rare in *N. virens* aquaculture (greater than marketed size) and the results obtained may not be relevant in the defining optimal aquaculture feed requirements. Conversely, it may be important to take into account maturity of the worms. Mature worms will tend to weigh at least 8 g. Age is not very significant in terms of time of maturity as it can occur between 1 and 8 years (Brafield and Chapman, 1967) the which point the worms (mainly the males) cease feeding. No mature individuals of 8 g or more were observed during the present research (all appeared to be feeding) and this aspect was therefore not taken into account.

Feed intake was closely associated with weight gain for worms of all sizes, showing that *N. virens* efficiently incorporates nutrients and energy into body mass with little feed waste. Neuhoff, 1979, estimated assimilation efficiencies for *N. virens* to be around 80 % when fed a *Mytilus edulis* based feed. Nielsen et al., 1995, observed a maximum mean ingestion rate of up to 50 mg shrimp meat ind^{-1} day^{-1} for *N. virens* weighing 0.2 to 0.7 g, with a calorific intake of 36.5 to 113 J g^{-1} day^{-1} respectively.

Present data suggests worms of around 1 g can consume around 14 mg feed g^{-1} day^{-1} in the form of a dry pelleted diet; the shrimp meat used in the study by Nielsen may have had considerable amounts of water present which would explain a much higher ingestion rate in that study. In the current experiments, no worms with a mean weight significantly below 1 g were used as they were unavailable in sufficient quantities. However, it may be of interest to investigate feed intake and growth for small juvenile cultured worms in order to extend our knowledge of feed requirements for all farmed stages of *N. virens*.

These findings can be compared with fish, where dietary protein requirements are generally found to be higher during the juvenile stages when growth rate is much faster than for older, larger fish, as in the channel catfish (Page and Andrews, 1973) and the olive flounder (Kim et al., 2002).

Protein, energy, moisture and ash levels of the whole body were unchanged for worms of different sizes fed to satiation (Fig. 14). The average protein content was 101.41 mg g^{-1} and the average energy content was 4822 J g^{-1}. This was also seen in the shrimp, *Litopenaeus vannamei,* the energy and protein contents were independent upon shrimp weight and were on average 4.83 kJ g^{-1} and 173 mg g^{-1} body mass respectively (Davies et al., 2008), whereas in many fish species, protein content remains stable while energy content increases with size (Lupatsch et al., 2003, Xie et al., 1997). In the case of salmonids, it has been shown that protein content is determined solely by fish size and not affected by growth rate or diet (Shearer, 2006). The relatively stable energy values across a range of sizes may be due to the inability of *N. virens* to store lipids in the body due to the lack of discrete organs for the storage of lipids or adipose tissue (Pocock et al., 1971); lipids are most commonly found in the body wall, coelomic fluid and gut tissues. Reserve lipids are likely to be used to provide energy for maturation and developing gametes, worms did not reach sexual maturity in this study and this may perhaps be the reason explaining why no changes in energy levels were noticed. The average nutrient level was therefore used as a standard from which the potential nutrient gain per day could be calculated for any given sized worm.

Data sets from rationing experiments (Experiments 1, 2, 3 and 5) were used to calculate the highest protein and energy gains relative to the amount fed. The resulting linear equations were then used to predict the amount of nutrient gain relative to intake as well as identifying the maintenance ration for both nutrients. As reported in Chapter 3, excess feeding above the level necessary for high growth resulted in lower nutritional gains. Under these circumstances, the worms were not utilising excess protein and energy and were instead excreting it; these data points were hence not used in the current analysis as they did not reflect the highest potential nutrient gains of the animals.

The energy maintenance requirement was 18 J day^{-1} per worm (Fig. 16); for protein, the requirement was 0.19 mg day^{-1} per worm (Fig. 15). These are the values at which *N. virens* will not show any nutritional gains or losses. According to Kay and Bradfield, 1973, the energy maintenance requirements for *N. virens* weighing 1.5 to 4 g fed *Nephtys hombergii* was approximately 30 J g^{-1} day^{-1}; these values are low relative to the present research. The differences may be due to the very different feed source, i.e. live polychaetes in contrast with dry pellets or perhaps the experimental conditions; Kay and Bradfield, 1973, reared *N. virens* in glass tubes subjected to complete darkness. The ragworm used in that research also originated from the wild (Southend-on-Sea, UK). Maintenance values derived from the present research are probably more valid in the context of polychaete farming; animals were reared in conditions and receiving feed similar to existing polychaete farms.

Maintenance protein requirements for shrimp *Litopenaeus vannamei* were found to be in the range of 1.8-3.8 mg protein g^{-1} body weight day^{-1} for juveniles and 1.5-2.1 mg protein g^{-1} body weight day^{-1} for subadult shrimp (Kureshy and Davis, 2002). In the case of catfish *Clarias batrachus*, protein maintenance requirements were found to be lower, with 0.942 mg protein g^{-1} body weight day^{-1} (Hassan and Jafri, 1994); the values found for *N. virens* were much lower with 0.19 mg protein day^{-1}. *N. virens* may have low protein requirements due to its more sedentary, infaunal lifestyle; fish and crustaceans generally lead a more active life and thus require higher levels of nutrients

to maintain their metabolic activities. The maintenance requirements will allow for worms to be kept at a stable market value but more importantly aid in understanding the metabolic needs and establishing daily rations for *N. virens*.

Table 21 also confirms the results in Chapter 4 regarding protein:energy ratio. Previous results found an optimum protein:energy ratio of 22.3 to 25.8 mg kJ^{-1}; following the bioenergetic modelling in Table 21, protein:energy ratio for worms weighing between 1 and 14 g was between 23.2 and 20.4 mg kJ^{-1}.

Based on the nutritional requirements of juvenile *N. virens* derived for different sizes (Table 21), feed formulations were devised which provide suitable energy and protein levels while minimizing waste to the environment, as seen in Table 22. For the protein level, this is calculated by obtaining the ratio of dietary protein level required relative to the amount that can be consumed.

For example, a 1 g worm requires 4.41 mg day^{-1} protein and has a voluntary feed intake of 12.6 mg day^{-1}. All the 4.41 mg protein has to be incorporated in the 12.6 mg of feed (4.41 / 12.6) which results in a feed with a dietary protein content of 350 mg g^{-1}.

The same calculations are used for the energy level formulations; the energy requirement J day^{-1} is divided by voluntary feed intake mg day^{-1}.
190 J / 12.6 mg = 15.07 kJ energy g^{-1} feed.

FCR is deduced by relating the voluntary feed intake mg day^{-1} to the subsequent weight gain in mg day^{-1}.

Dietary protein and energy content stayed relatively the same and only decreased slightly for larger worms. The predicted FCR also stayed low, between 0.84 and 1.5. Ad libitum feed intake rose as the worms become larger; larger worms are able to consume more feed relative to their size than smaller worms (Table 22). Table 22 demonstrates the feed formulations for different sized worms, although for practical purposes, one feed formulation would suffice in conjunction with changing feeding rates.

Table. 22. Possible feed formulations and anticipated performance for N. virens, weight 1-14 g, based on protein and energy requirements.

Worm size (g)	Feed composition g^{-1}		FCR	Feed intake % day^{-1} $biomass^{-1}$
	Protein mg	Energy kJ		
1	351	15.07	0.84	1.3
3	272	13.4	1.07	1.8
5	245	10.45	1.2	2.1
7	229	11.26	1.29	2.4
10	208	10.20	1.39	2.7
12	198	9.71	1.45	2.8
14	196	9.62	1.5	2.9

CHAPTER 6

Effects of Alternative Feed Sources on the Culture Performance and Nutritional Quality of *Nereis virens*.

6.1 Introduction

Development of non-fish based sustainable feeds is one of the most pressing areas of research in aquaculture. Farmed polychaetes provide a good source of protein, lipids and essential fatty acids but can only be recognized as sustainable alternatives to industrial fish meal and fish oil if these raw materials are not included in their diet. At the outset of this research, *N. virens* farmed by the industry sponsor received a conventional formulated feed containing both fish meal and fish oil. A significant element of the commercial value of *N. virens* as an aquaculture feed is believed to lie in its HUFA content, since eicosapentaenoic acid, EPA (20:5n-3) and docosahexaenoic acid, DHA (22:6n-3) are very important for the healthy development of marine fish and crustaceans (Rainuzzo et al., 1997; Watanabe, 1982; Sargent et al., 1989). Marine fish and crustaceans are unable to synthesise these acids *de novo* and require them to be present in their diet. Experiments involving non-marine based feeds in a polychaete farm have not been published in scientific literature and therefore the ability of *N. virens* to elongate and desaturate shorter chain fatty acids to EPA and DHA is unknown.

N. virens in the wild has a wide range of feeding strategies including omnivorous, carnivorous and detritivorous behaviours. Many different food items have been found in the gut such as vegetable and animal detritus, diatoms and polychaetes including *N. virens* (Caron et al., 2004). *N. virens* can also adjust its digestive enzymes depending on the food available, for example, cellulase occurs in the gut of *N. virens* and its production is induced by the presence of algae (Bock and Mayer, 1999). Being an opportunistic and adaptable species with regard to feed sources, it may be expected that many diets will be not only palatable to the species, but also promote suitable growth and a healthy development.

There are many alternate sources of feeds that can be investigated as diets such as animal by-products and vegetable proteins. Animal wastes include poultry feathers, viscera, skin, blood meal, bone meal and other industrial by products. The advantage of

these feeds is the protein content and low cost; potential disadvantages include lack of correct amino acid balances or essential fatty acid composition (Naylor et al., 2000). Vegetable based feeds such as soy and wheat can also provide adequate protein levels but lack n-3 fatty acids present in fish meal as well as certain amino acids and can contain antinutritional factors (Francis et al., 2001). While marine fish species lack the ability to convert fatty acids to EPA or DHA, possibly due to a lack of the enzyme Δ5-desaturase, many freshwater fish species are able to do so (Glencross et al., 2009).

Although there have not been any published studies on the ability of *N. virens* to convert fatty acids to EPA or DHA, some research has been done on a close relative, *N. diversicolor*. When fed soy and pollen, it was found that this species was, to a certain extent, able to synthesize EPA and DHA *de novo* (Fidalgo e Costa et al., 2000). *N. diversicolor* has a different feeding strategy to *N. virens*; it is a facultative filter feeder and can obtain its nutritional requirements from a diet of phytoplankton if the concentration is high enough (Nielsen et al., 1995). Filter feeding has not been found to be a strategy used by *N. virens*, yet studies by Papaspyrou et al., 2005, suggest that *N. virens* can take advantage of phytoplankton by trapping particles in the mucous lining the burrow. However, *N. virens* ventilates its burrow 5 to 10 times less than *N. diversicolor* (Christensen et al., 2000), which shows that the latter species is more adapted to take advantage of food particles in the water column.

Many marine invertebrates, including polychaetes, are able to uptake free fatty acids from seawater such as oleic, palmitic, stearic and linoleic acids. For example, the polychaete *Nainereis dendritica*, can concentrate palmitic acid by a factor of 25 over the medium (Testerman, 1972). Testerman (1972) also demonstrated that other marine polychaetes as well as oligochaetes, echiuroid worms and echinoderms can similarly uptake free fatty acids. Although the amount of free fatty acids that marine invertebrates can uptake is high, it is likely that the levels in the environment are too low to support more than a few percent of the organism's metabolism. Testerman, 1972, estimated that the total uptake of long chain free fatty acids could support 16 % of the polychaete *Stauronereis rudolphi* oxidative metabolism.

N. virens is grown and harvested by the industrial sponsor of this research in large outdoor raceways which develop their own ambient fauna and flora over time. The physical parameters are rarely tampered with and the raceways are subject to changes in temperature, salinity and weather conditions. The raceway environment has not been described in detail, but by observation, the main macrofauna present is generally crustacean amphipods which are free swimming and also burrowing in the sand. The water also appears to contain significant amounts of phytoplankton and protist communities as seen by the vivid green, opaque appearance of the water. Previous studies on the uptake of fatty acids from the environment by marine annelids such as *Stauronereis rudolphi* (Testerman, 1972) and *Glycera americana* (Ferguson, 1982), suggest that *N. virens* may be able to utilize fatty acids in the form of phytoplankton and protists such as heterotrophic ciliates and flagellates when ventilating or maintaining their burrow.

The aims of the current experiments were to ascertain the ability of *N. virens* to survive, grow and maintain a suitable nutritional profile using feeds formulated without marine ingredients, including preliminary investigation of whether *N. virens* can elongate and desaturate fatty acids such as 18:3n-3 from terrestrial sources to n-3 HUFA. The research included comparison of the non-marine test feeds under laboratory versus farm conditions in which the polychaete worms have access to additional nutrient sources such as naturally occurring microalgae.

6.2 Materials and Methods

6.2.1 Experiment 6: Effects of Different Feed Sources on the Nutritional Quality of *N. virens.*

This experiment, carried out in the wet laboratory, consisted of 3 tub replicates for each of 4 feeds (poultry meal, soy meal, fish meal and commercial control), resulting in 12 tubs containing 20 worms each. All tubs were fed slightly to excess and leftovers counted and siphoned after 24 hours (weight of pellets taken beforehand). Experiment duration was 50 days after which worms from one replicate per feed treatment was analysed for fatty acid composition, together with samples of each feed. Worms from the remaining two replicates were subject to proximate analysis (protein, energy and lipid content) along with their respective feeds. Water temperature was regulated to 17.5±1.5°C and salinity to 29±0.5 ‰.

Feed formulations and proximate compositions are listed in Table 23.

Table. 23. Formulation and proximate composition of various trial Dragon Research Ltd feeds: poultry meal, soy meal and fish meal as well as a commercial fish meal control diet used in Experiment 6. Pellet size: 2.5-3 mm diameter.

Feed ingredients %	**Poultry meal**	**Soy meal**	**Fish meal**	**Commercial Control**
Soyflour protein	-	50	-	
Fish meal	-	-	50	
Poultry meal	50	-	-	
Wheat feed	41	41	41	
Dried seaweed	2	2	2	
Vitamin premix	2	2	2	
Vegetable oil	-	5	5	
Used vegetable oil	5	-	-	
Unknown				Unknown quantities of soybean meal, wheatfeed, wheat, fish oil and fish meal
Proximate composition				
Dry matter %	91.47	93.54	94.07	91.80
Protein %	35.60	33.13	42.12	41.18
Lipid %	11.30	6.60	8.60	11.4
Ash %	10.74	5.62	12.64	7.74
Energy kJ g^{-1}	19.54	19.15	18.00	20.46
Protein:energy mg kJ^{-1}	18.22	17.28	23.39	20.14

Pellet size of the experimental feeds was manufactured to be 2.5-3.0 mm diameter, to match that of the commercial control feed. The control feed, not being manufactured at Dragon Research Ltd., had a largely unknown ingredient content (closed formula).

6.2.2 Experiment 7: Effects of Different Feed Sources both in the Laboratory and in an Outdoor Polychaete Farm.

This experiment was split between two locations, i.e. the wet laboratory used for the previous experiments and at Dragon Research Ltd. polychaete farm in Port Talbot. The field location was a purpose built concrete raceway, around 50 m long, 10 m wide and 1 m deep, containing approximately 40 cm depth of local beach sand. A paddle

aerator in the middle of the raceway oxygenated the water and provided directional water flow. Water depth was around 60 cm above the sediment level. As this was an outdoor site and the raceways were relatively shallow, substantial diurnal and seasonal variations occurred in water temperature. Salinity was also variable associated with variations in rainfall and evaporation. These effects could be mitigated to a certain extent by water exchanges provided by a water pump linking the raceways in the farm to the nearby sea.

Three different practical diets were tested, a high protein diet based on fish meal (HPF), a high protein diet based on plant sources and blood meal (HPP) and a low protein fish meal based diet (LPF), their formulation and composition is listed in Table 24.

Table. 24. Formulation and proximate composition for different diets used in Experiment 7. HPF = High protein fish meal, HPP = High protein plant and blood meal, LPF = Low protein fish meal, as well as organic matter sampled from water at the polychaete farm.

Ingredients	**HPF**	**HPP**	**LPF**	**Organic matter**
Salmon Pro hydro (*2)	14.00	-	6.00	
Distiller Dried Grains	16.20	18.83	38.04	
Calcium sulphate	2.59	2.96	-	
Soya protein concentrate	10.03	22.14	-	
Di-Calcium Phosphate	0.14	-	-	
Hi Pro Soya bean meal	25.00	25.00	-	
Spray Dried Haem (APC)	6.09	5.86	-	
Vegetable oil (rapeseed)	2.08	4.00	0.95	
Wheat Flour	23.87	21.21	34.07	
Tapioca Starch	-	-	20.95	
Proximate composition				
Dry matter %	91.22	88.05	88.53	10.37
Protein %	38.52	35.17	18.59	
Lipid %	8.22	7.35	7.98	1.72
Ash %	6.10	5.60	17.51	0.93
Energy kJ g^{-1}	20.15	19.95	15.79	
Protein:energy mg kJ^{-1}	19.11	17.64	11.78	

Each diet was tested via 3 tub replicates at each location; 4 tubs of unfed worms were also included at each location:

Wet laboratory: 3 x 12 worms – HPF
3 x 12 worms – HPP
3 x 12 worms – LPF
3 x 6 worms – Starvation
1 x 12 worms – Starvation

Farm Raceway: 3 x 12 worms – HPF
3 x 12 worms – HPP
3 x 12 worms – LPF
3 x 6 worms – Starvation
1 x 12 worms – Starvation

At the farm site, high mesh barriers, standing 20 cm higher than the tubs, bordered the inside of the tubs in order for the raceway water to flow inside but to prevent the worms from migrating out. The tubs were placed on the surface of the existing sediment in the raceway. Another layer of mesh was placed on top of the system blocking the opening in order to prevent feed destined for the farmed worms in the raceway getting into the experimental tubs.

Worms at both the wet laboratory and the field locations were fed three times per week. Feed was offered at the maximum consumption rate observed in previous experiments. Each feed type was pre-weighed into vials and a sprinkling offered on each occasion. The water at the field location was very dark and opaque (microalgae bloom), preventing observation of any leftover feed, therefore it was decided not to siphon away uneaten food at either location.

Mesh of the tubs in the raceways was scrubbed with a brush regularly to remove build up of algae and epiphytes which would impede water flow. Trial duration was 38 days, after which worms from the raceway were brought back to the wet laboratory and left to

purge for two days in seawater from the farm. Wet laboratory worms were also purged at the same time. Ragworm analyses were carried as described in Materials and Methods. Additionally, a 2 g sample of worms was collected from each tub replicate immediately after harvesting and frozen for subsequent fatty acid analysis.

Sea water from the raceways was also sampled to quantify the fatty acid composition of organic matter. Refrigerated samples were shaken and placed in a Hettich Universal 320 centrifuge in 4 x 50 ml pre weighed containers, then centrifuged for 15 minutes at 4000 rpm. After centrifugation, the supernatant was decanted and another 50 ml water was added. Centrifuge cycles were repeated until a quantity of 250 ml per vial, or a total of 1000 ml was reached. At the end of this, 20 ml of freshwater was added and centrifuged as a means of rinsing away any residual salt. Supernatant was again discarded, the vial with sample residue at bottom weighed and then frozen until analysis. Sample weight was calculated by subtracting the container with residue from the empty container weight. 1000 ml of seawater resulted in around 1 g of wet sample.

The data for fed groups were analysed by two-way ANOVA to take into account the combination of different diets and test locations. Data for the 2 starvation groups were analysed separately by means of a t-test.

6.3 Results

6.3.1 Experiment 6 - Effects of Different Feed Sources on *N. virens.*

The performance of *N. virens* fed poultry, soy and fish meal as well as a control is summarised in Table 25. All diets resulted in similar mean cumulative survival rates, ranging between 73.3 and 76.7 %, although some mortalities occurred beneath the sediment and were hence unaccounted for until the end of the experiment. The worms more than doubled their weight over 50 days with weight gains of 86.9 to 105.5 mg ind^{-1} day^{-1}; there were no statistically significant differences in SGR among diets, with mean values ranging from 1.22 to 1.61 % per day. No significant differences were found either in the amount of feed consumed. However, towards the middle and end of the experiment, harpacticoid copepods were noticed on the surface of the sediments and following this observation, their numbers seemed to increase and were seen consuming feed pellets. The worms were calculated to have consumed 1.40 to 1.49 % of their body weight daily on average.

Table. 25. Performance of N. virens fed different formulated diets: soy meal, poultry meal, fish meal and a commercial fish meal based control. Statistical similarities or differences are expressed by superscripted lower case letters for each data point ($p<0.05$). Mean values ± SD.

	Control	**Soy meal**	**Fish meal**	**Poultry meal**
Initial weight g	3.87 ± 0.16	3.95 ± 0.13	4.02 ± 0.08	3.90 ± 0.0
Final weight g	9.15 ± 1.18	7.83 ± 0.86	9.92 ± 1.13	8.77 ± 0.81
Weight gain mg ind^{-1} day^{-1}	94.39^a ± 19.90	69.30^a ± 16.74	105.48^a ± 19.87	86.93^a ± 14.49
Survival %	75.00^a ± 8.66	76.67^a ± 11.55	75.00^a ± 17.32	73.33^a ± 5.77
Feed intake mg ind^{-1} day^{-1}	86.34^a ± 8.63	82.90^a ± 9.73	88.71^a ± 17.34	86.21^a ± 7.67
Feed intake % day^{-1}	1.45^a ± 0.04	1.49^a ± 0.14	1.40^a ± 0.20	1.47^a ± 0.08
FCR	0.93^a ± 0.12	1.24^a ± 0.30	0.84^a ± 0.01	1.00^a ± 0.09
SGR % day^{-1}	1.53^a ± 0.22	1.22^a ± 0.23	1.61^a ± 0.21	1.44^a ± 0.17

Values for the proximate composition of *N. virens* at the start and end of the experiment can been in Table 26. Ash and dry matter contents increased for all worms relative to the initial samples ($p<0.05$), although there were not any significant differences among feed types at the end of the experiment.

Table. 26. Proximate composition per g live weight N. virens fed different diets: soy meal, poultry meal, fish meal and a commercial fish meal control. Statistical similarities or differences are expressed by superscripted lower case letters for each data point ($p<0.05$). Mean values ± SD.

	Initial	**Control**	**Soy meal**	**Fish meal**	**Poultry meal**
Dry matter %	14.64	21.83^{a} ± 3.45	19.25^{a} ± 0.58	19.14^{a} ± 0.59	19.92^{a} ± 0.57
Ash %	1.57	3.50^{a} ± 1.24	3.41^{a} ± 0.39	3.34^{a} ± 0.12	4.94^{a} ± 0.34
Protein %	7.79	10.61^{a} ± 1.55	9.56^{a} ± 0.03	9.16^{a} ± 0.29	8.51^{a} ± 0.13
Lipid %	3.51	4.86^{a} ± 0.31	3.87^{b} ± 0.10	3.95^{b} ± 0.12	4.01^{ab} ± 0.28
Energy J g^{-1}	3579	5046^{a} ± 599	4254^{a} ± 319	4234^{a} ± 111	4048^{a} ± 166

There were no statistically significant differences in body protein and energy levels among feed treatments, nor any significant relationship between the amount of protein fed and the amount retained in the body (Table 27). Protein gain ranged from 1.42 to 1.93 mg g^{-1} day^{-1} and energy gain from 65.93 and 93.10 J g^{-1} day^{-1}.

Table. 27. Energy and protein efficiencies of N. virens fed different diets: soy meal, poultry meal, fish meal and a commercial fish meal based control feed. Statistical similarities or differences are expressed by superscripted lower case letters for each data point ($p<0.05$). Mean values ± SD.

	Control	Soy meal	Fish meal	Poultry meal
Protein consumed mg g^{-1} day^{-1}	5.65^a ± 0.16	5.28^a ± 0.48	6.26^a ± 0.89	6.61^a ± 0.35
Protein gain mg g^{-1} day^{-1}	1.93^a ± 0.66	1.51^a ± 0.17	1.79^a ± 0.12	1.42^a ± 0.07
PRE %	34.41^a ± 10.86	29.64^a ± 0.32	26.44^a ± 1.58	20.87^a ± 1.49
Energy consumed J g^{-1} day^{-1}	310.19^a ± 8.82	305.3^a ± 27.85	267.75^a ± 37.93	328.39^a ± 17.32
Energy gain J g^{-1} day^{-1}	93.10^a ± 27.53	65.93^a ± 5.07	83.16^a ± 7.20	69.15^a ± 9.20
ERE %	30.28^a ± 8.17	22.39^a ± 0.54	28.72^a ± 2.31	20.50^a ± 3.18

Fatty acid compositions of the feeds are summarised in Table 28 and of the worms in Table 29. The fish meal based experimental feed contained higher levels of DHA and EPA than those feeds based on soy meal or poultry meal. Arachidonic acid (ARA), 20:4n-6, content was low (<1 %) in all feed formulations, being almost undetectable in the soy meal-based feed. Levels of linolenic acid, 18:3n-3, and linoleic acid, 18:2n-6, were higher in all experimental feeds than in the commercial control.

In line with feed compositions, the mean body contents of linoleic acid and linolenic acid were higher among worms receiving the three experimental feeds compared to those receiving the commercial control feed. In all treatments, linoleic and

linolenic acids contributed a lower proportion of total fatty acids in the worm tissue than in the feeds.

Tissue ARA levels were low in all treatments, being undetectable in those worms receiving the poultry meal-based feed.

Tissue levels of DHA and EPA corresponded directly to dietary content, as expressed in significant ($p<0.05$) positive correlations between % DHA and EPA in tissue and in feeds (Fig. 17 and 18). Quantities of DHA and EPA (% total fatty acids) in worm tissue were in all cases lower at the end of the experiment than in the initial samples.

Table. 28. Fatty acid composition mg g^{-1} (% of fatty acids in brackets) of different experimental diets: soy meal, poultry meal, fish meal and a commercial fish meal control. Values represent averages of duplicate analyses.

Fatty acid	Control	Soy meal	Fish meal	Poultry meal
14:0	3.97 *(5.01)*	0.21 *(0.45)*	0.74 *(1.23)*	0.59 *(0.75)*
16:0	12.64 *(15.97)*	4.54 *(9.87)*	6.29 *(10.42)*	11.77 *(14.88)*
18:0	1.89 *(2.39)*	0.85 *(1.85)*	1.34 *(2.22)*	3.57 *(4.51)*
16:1n-9	0.00	0.00	0.09 *(0.15)*	0.20 *(0.25)*
16:1n-7	3.67 *(4.64)*	0.25 *(0.55)*	1.08 *(1.79)*	1.91 *(2.42)*
18:1n-9	10.60 *(13.39)*	20.66 *(44.92)*	24.59 *(40.77)*	32.91 *(41.61)*
18:1n-7	1.76 *(2.22)*	1.19 *(2.59)*	1.59 *(2.64)*	1.87 *(2.37)*
20:1n-9	6.11 *(7.72)*	0.63 *(1.37)*	1.63 *(2.70)*	0.85 *(1.07)*
18:2n-6	7.00 *(8.85)*	13.35 *(29.02)*	12.34 *(20.45)*	18.73 *(23.68)*
20:2n-6	0.21 *(0.26)*	0.06 *(0.13)*	0.12 *(0.20)*	0.14 *(0.18)*
20:4n-6	0.41 *(0.51)*	0.06 *(0.14)*	0.28 *(0.46)*	0.46 *(0.59)*
18:3n-3	1.35 *(1.70)*	3.03 (6.58)	3.38 *(5.61)*	3.69 *(4.66)*
20:3n-3	0.16 *(0.20)*	0.00	0.00	0.00
20:5n-3	5.18 *(6.54)*	0.05 *(0.11)*	1.39 *(2.30)*	0.38 *(0.48)*
22:6n-3	8.45 *(10.67)*	0.27 *(0.58)*	2.17 *(3.60)*	0.47 *(0.59)*

Table. 29. Fatty acid composition mg g^{-1} of N. virens live weight (% of fatty acids in brackets) of N. virens fed different experimental diets: soy meal, poultry meal, fish meal and a commercial fish meal control.

Fatty acid	Initial	Control	Soy meal	Fish meal	Poultry meal
14:0	0.39 *(1.67)*	0.40 *(1.68)*	0.21 *(0.82)*	0.19 *(0.90)*	0.18 *(0.79)*
16:0	3.93 *(16.69)*	4.40 *(18.29)*	4.19 *(16.40)*	3.35 *(16.20)*	3.84 *(16.76)*
18:0	0.64 *(2.70)*	0.65 *(2.70)*	0.68 *(2.65)*	0.51 *(2.46)*	0.68 *(2.95)*
16:1n-9	0.10 *(0.43)*	0.12 *(0.49)*	0.18 *(0.70)*	0.13 *(0.63)*	0.17 *(0.76)*
16:1n-7	1.36 *(5.77)*	1.29 *(5.36)*	1.05 *(4.10)*	0.72 *(3.48)*	1.00 *(4.36)*
18:1n-9	1.88 *(7.99)*	1.99 *(8.28)*	3.69 *(14.43)*	2.93 *(14.16)*	3.90 *(17.04)*
18:1n-7	1.49 *(6.34)*	1.57 *(6.54)*	1.36 *(5.33)*	1.07 *(5.19)*	1.22 *(5.35)*
20:1n-9	1.16 *(4.94)*	1.28 *(5.33)*	0.97 *(3.79)*	0.99 *(4.79)*	0.96 *(4.18)*
18:2n-6	0.89 *(3.79)*	0.99 *(4.11)*	2.38 *(9.29)*	1.43 *(6.93)*	2.09 *(9.11)*
20:2n-6	1.14 *(4.82)*	1.16 *(4.82)*	2.15 *(8.40)*	1.72 *(8.33)*	1.88 *(8.23)*
20:4n-6	0.29 *(1.24)*	0.25 *(1.04)*	0.52 *(2.03)*	0.29 *(1.42)*	0.00
18:3n-3	0.29 *(1.24)*	0.28 *(1.17)*	0.69 *(2.69)*	0.49 *(2.37)*	0.52 *(2.27)*
20:3n-3	0.07 *(0.31)*	0.07 *(0.29)*	0.11 *(0.45)*	0.10 *(0.50)*	0.09 *(0.41)*
20:5n-3	2.63 *(11.17)*	2.14 *(8.92)*	1.59 *(6.22)*	1.57 *(7.57)*	1.33 *(5.79)*
22:6n-3	1.64 *(6.94)*	1.26 *(5.26)*	0.40 *(1.58)*	0.74 *(3.57)*	0.34 *(1.49)*

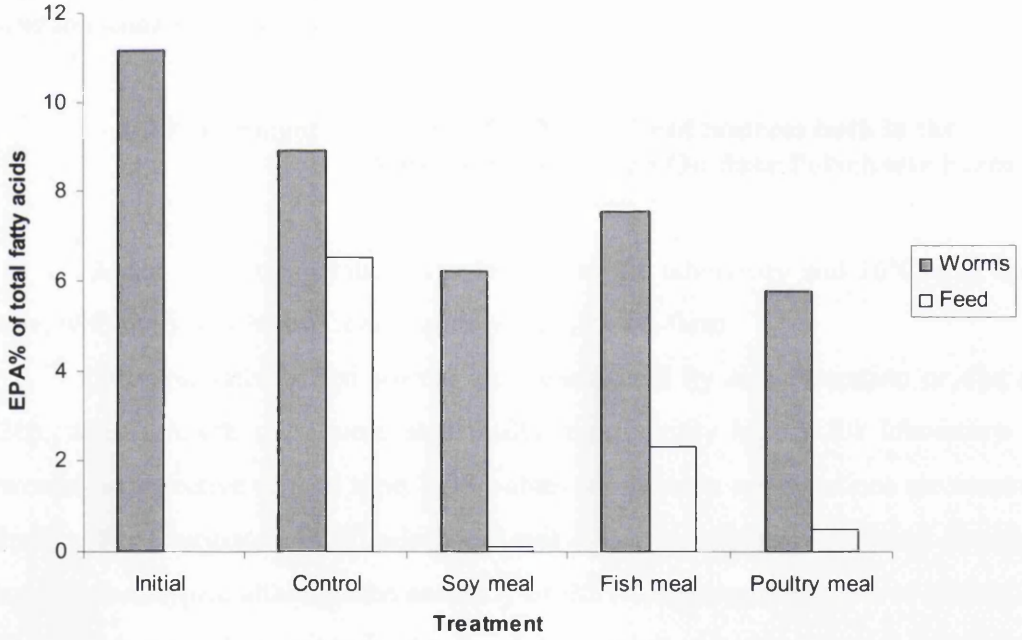

Fig. 17. Level of EPA in % total fatty acids of N. virens relative to the amount of fatty acid in the feed (soy meal, poultry meal, fish meal and a commercial fish meal based feed control) and to initial worm samples.

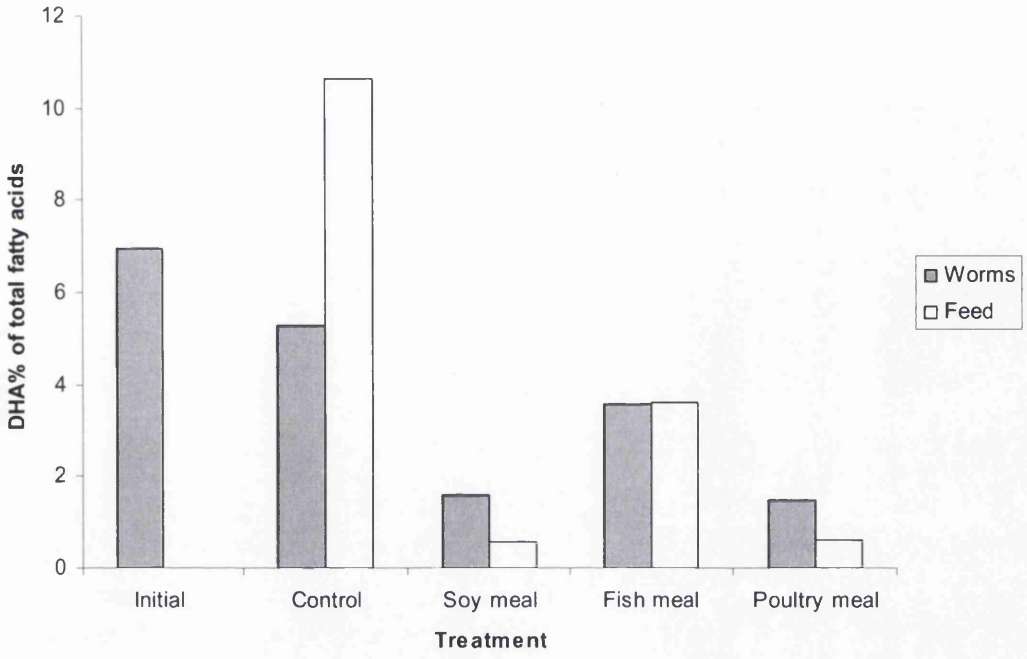

Fig. 18. Level of DHA in % total fatty acids of N. virens relative to the amount of fatty acid in the feed (soy meal, poultry meal, fish meal and a commercial fish meal control) and to initial worm samples.

6.3.2 Experiment 7 - Effects of Different Feed Sources both in the Laboratory and in an Outdoor Polychaete Farm.

Mean water temperature was 18°C±1 in the laboratory and 16°C±4 at the field site, with markedly higher temperature variability on-farm.

Survival rates of fed worms were unaffected by either location or diet (Table 30), while growth rates were statistically significantly higher for laboratory reared worms, irrespective of feed type. SGR values for all diets and locations are summarised in Fig 19. Calculated FCR values did not differ significantly between locations or among feed types, although the accuracy of the FCR measurements was limited by the inability to quantify uneaten food at the field location or to detect any mortalities before the end of the experiment. Calculated values for protein and energy efficiencies are subject to the same constraints, being derived from feed intake data.

There were no statistically significant differences for any performance parameters between locations for unfed worms (Table 31).

Table. 30. Performance of N. virens fed different diets (HPF = High protein fish meal, HPP = High protein plant and blood meal, LPF = Low protein fish meal) at two locations: laboratory and polychaete farm. Mean values ± SD.

	Laboratory			**Farm**		
	HPF	**HPP**	**LPF**	**HPF**	**HPP**	**LPF**
Initial weight g	4.90 ± 0.26	5.31 ± 0.23	5.17 ± 0.27	4.89 ± 0.33	4.91 ± 0.42	4.68 ± 0.23
Final weight g	8.34 ± 1.28	8.04 ± 0.46	6.65 ± 1.38	5.62 ± 0.38	6.40 ± 1.05	5.22 ± 0.30
Weight gain mg ind^{-1} day^{-1}	81.9 ± 29.40	65.1 ± 6.64	35.3 ± 28.07	17.3 ± 8.52	35.4 ± 16.87	12.9 ± 8.30
Survival %	75.00 ± 22.05	77.78 ± 17.35	63.89 ± 20.97	66.67 ± 36.32	75.00 ± 16.67	69.44 ± 26.79
Feed offered mg ind^{-1} day^{-1}	87.97 ± 30.53	82.12 ± 20.48	104.5 ± 38.41	128.98 ± 101.43	84.85 ± 19.3	96.66 ± 31.68
Feed intake % day^{-1}	1.36 ± 0.37	1.27 ± 0.38	1.77 ± 0.48	2.40 ± 1.76	1.55 ± 0.50	1.97 ± 0.68
FCR	1.08 ± 0.02	1.28 ± 0.40	7.94 ± 10.08	7.81 ± 3.97	2.73 ± 1.30	14.83 ± 17.24
SGR % day^{-1}	1.25 ± 0.34	0.99 ± 0.07	0.57 ± 0.42	0.33 ± 0.16	0.62 ± 0.22	0.26 ± 0.16

Table. 31. Performance of unfed N. virens at two locations: laboratory and polychaete farm. Statistical similarities or differences are expressed by superscripted lower case letters for each data point ($p<0.05$). Mean values ± SD.

	Laboratory Unfed	**Farm Unfed**
Initial weight g	5.01^a ± 0.23	4.55^a ± 0.24
Final weight g	4.12^a ± 0.55	4.50^a ± 0.90
Weight gain mg ind^{-1} day^{-1}	-21.3^a ± 15.73	-1.1^a ± 15.73
Survival %	64.58^a ± 21.92	91.67^a ± 11.79
SGR % day^{-1}	-0.48^a ± 0.36	-0.10^a ± 0.27

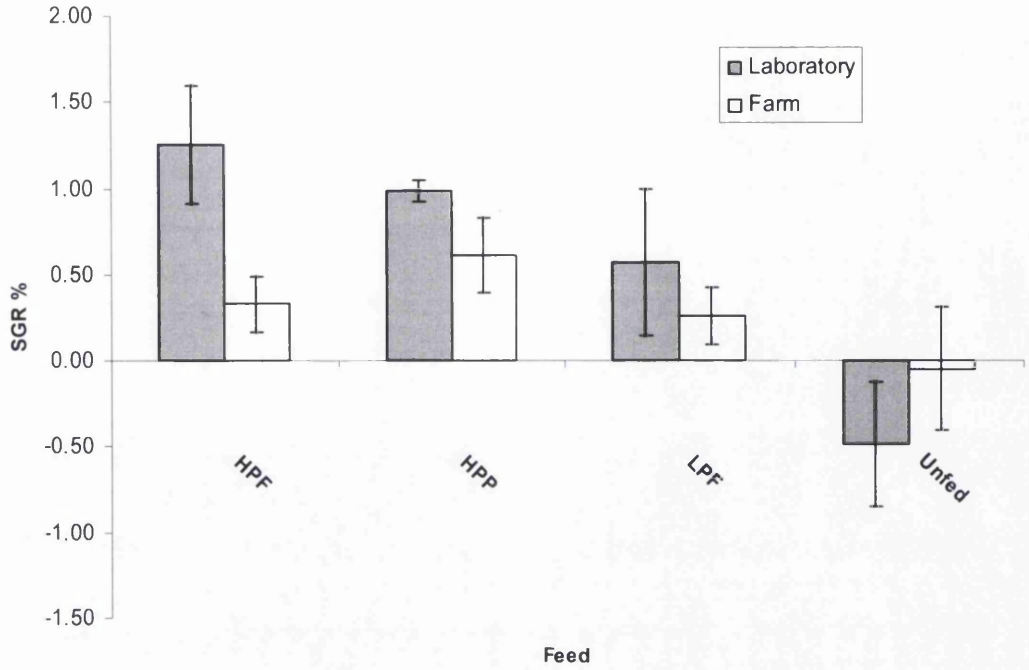

Fig. 19. SGR % day^{-1} of N. virens subjected to laboratory and farm environments when fed different diets and under starvation conditions. HPF = High protein fish meal, HPP = High protein plant and blood meal, LPF = Low protein fish meal. Mean values ± SD.

The proximate compositions of the worms are listed in Tables 32 and 33. Fed worms reared in the laboratory contained statistically significantly greater percentages of dry matter and ash than those reared on farm ($p<0.05$). Location also had a statistically significant effect on the energy content of fed worms ($p<0.05$). Feed type did not significantly affect dry matter, ash or energy content, however protein levels were significantly affected by feed type ($p<0.01$). Unfed groups on farm and in the laboratory did not differ significantly from each other in terms of dry matter, ash, protein or energy content.

Table. 32. Proximate composition per g live weight N. virens fed different diets (HPF = High protein fish meal, HPP = High protein plant and blood meal, LPF = Low protein fish meal) at two locations: laboratory and polychaete farm. Mean values ± SD.

		Laboratory			Farm		
	Initial	**HPF**	**HPP**	**LPF**	**HPF**	**HPP**	**LPF**
Dry matter %	18.60	19.06 ± 0.62	18.79 ± 0.11	18.97 ± 0.57	18.98 ± 1.02	17.78 ± 0.59	17.90 ± 0.50
Ash %	1.97	1.50 ± 0.03	1.53 ± 0.03	1.67 ± 0.12	1.72 ± 0.07	1.73 ± 0.11	1.66 ± 0.11
Protein %	9.53	9.59 ± 0.25	9.63 ± 0.18	9.06 ± 0.35	9.85 ± 0.43	8.75 ± 0.33	8.72 ± 0.26
Lipid %	4.6	4.17 ± 12.2	4.37 ± 2.02	3.67 ± 2.77	4.29 ± 2.76	3.9 ± 2.24	4.16 ± 3.12
Energy J g^{-1}	4339	4411 ± 212	4363 ± 39	4366 ± 188	4390 ± 260	4126 ± 149	4020 ± 185

Table. 33. Proximate composition per g live weight of unfed N. virens at two locations: laboratory and polychaete farm. Statistical similarities or differences are expressed by superscripted lower case letters for each data point ($p<0.05$). Mean values ± SD.

	Laboratory Unfed	**Farm Unfed**
Dry matter %	16.62^{a} ± 1.35	17.83^{a} ± 0.35
Ash %	1.69^{a} ± 0.41	1.68^{a} ± 0.09
Protein %	9.27^{a} ± 0.72	9.37^{a} ± 0.31
Energy J g^{-1}	3728^{a} ± 225	4004^{a} ± 92

Calculated energy and protein efficiencies for fed and unfed worms at each location are listed in Tables 34 and 35. Both location ($p<0.001$) and diet ($p<0.001$) had statistically significant effects on protein gain mg g^{-1} day^{-1} and PRE for fed worms (Table 34). Protein gain and PRE of the worms both corresponded to the protein content of the feeds, with consistently higher values for these parameters in the laboratory than on farm.

Calculated energy gain and ERE were also significantly affected by both location ($p<0.001$) and diet ($p<0.05$) for fed worms, being lowest for worms reared on farm and receiving feeds with low protein content (Table 34).

There were no statistically significant effects of location on the calculated energy loss or ERE of unfed worms.

Table. 34. Energy and protein loss of N. virens fed different diets (HPF = High protein fish meal, HPP = High protein plant and blood meal, LPF = Low protein fish meal) at two locations: laboratory and polychaete farm. Mean values ± SD.

	Laboratory			Farm		
	HPF	**HPP**	**LPF**	**HPF**	**HPP**	**LPF**
Protein consumed mg g^{-1} day^{-1}	5.73 ± 1.54	5.33 ± 1.59	7.42 ± 2.01	10.09 ± 7.41	5.44 ± 1.77	3.66 ± 1.27
Protein gain mg g^{-1} day^{-1}	1.23 ± 0.30	0.98 ± 0.06	0.41 ± 0.32	0.39 ± 0.16	0.37 ± 0.16	0.04 ± 0.11
PRE %	21.53 ± 1.65	19.24 ± 4.80	5.98 ± 5.09	4.55 ± 1.59	7.70 ± 4.46	1.61 ± 2.85
Energy consumed J g^{-1} day^{-1}	274.7 ± 74.12	253.1 ± 75.59	279 ± 75.52	484.3 ± 355.7	308.5 ± 100.5	311.2 ± 108
Energy gain J g^{-1} day^{-1}	56.95 ± 9.80	43.85 ± 1.98	25.34 ± 16.58	15.60 ± 8.83	20.99 ± 9.00	3.18 ± 3.23
ERE %	21.09 ± 2.47	18.29 ± 5.07	9.87 ± 7.80	3.49 ± 0.83	7.60 ± 4.38	1.45 ± 1.85

Table. 35. Energy and protein loss of unfed N. virens at two locations: laboratory and polychaete farm. Statistical similarities or differences are expressed by superscripted lower case letters for each data point ($p<0.05$). Mean values ± SD.

	Laboratory Unfed	**Farm Unfed**
Protein loss mg g^{-1} day^{-1}	-0.51^a ± 0.45	-0.08^a ± 0.41
Energy loss J g^{-1} day^{-1}	-34.05^a ± 17.70	-9.86^a ± 16.95

Fatty acid compositions of the Experiment 7 feeds and worms are described in Tables 36 and 37 respectively. The content of n-3 HUFA in the experimental feeds was low in all cases, ranging from 0.0 to 1.1 mg g^{-1} for DHA and 0.0 to 0.5 mg g^{-1} for EPA. Levels of DHA and EPA within this range were also found in lipids extracted from suspended organic matter at the farm site. ARA was only detected at significant levels in this organic matter, not in the formulated feeds. The feed contents of linoleic acid, 18:2n-6, and oleic acid, 18:1n-9, varied greatly depending on formulation, being highest in the LPF feed.

Tissue DHA contents were similar for all fed worms regardless of location or feed composition, ranging from 0.33 to 0.56 mg g^{-1} tissue wet weight (Table 37, Fig. 21). Location and diet both had a statistically significant effect on tissue EPA and ARA content ($p<0.001$ and $p<0.05$ respectively). Tissue EPA contents showed relatively little variation among feed types, but were consistently higher among laboratory reared than farm reared worms (Table 37, Fig. 20). Tissue contents of ARA were also consistently higher among laboratory than farm reared worms. Detectable levels of these three essential fatty acids were recorded in all *N virens* tissue samples, even where undetectable in the respective formulated feeds.

Tissue contents of linoleic acid, 18:2n-6, were low in all fed groups (mean 1.3 mg g^{-1}), despite the highly variable content of this fatty acid in the different

experimental feeds. The oleic acid, 18:1n-9, contents of worms was also relatively low and consistent among feed groups compared to feed composition.

In order to extract organic matter from the polychaete farm raceway water, approximately 1 litre of water was needed to collect 1 g of wet material by centrifugation. The fatty acid profile showed high levels of ARA, EPA and DHA: 0.33, 1.34 and 0.45 mg g^{-1} wet weight respectively.

A calculation can be made regarding the total polychaete lipid in relation to the total polychaete biomass taking into account growth of the animals. This was done using the following equation for ARA, EPA and DHA:

Total worm lipid = no. of worms x wet mean weight of worms x lipid % of the total biomass

Following a further calculation on the actual fatty acid amount given to the worm it is possible to then estimate if any fatty acid gains occurred in the worms.

Results revealed that no actual net gain occurred in the worms in either the lab or the farm for any of the three fatty acids with the exception of ARA in the case of the laboratory HPP worms. There was an increase from 0.04 % of total ARA in the worm biomass to 0.054 %, taking into account that there was an absence of ARA in the HPF feed. There is therefore a high possibility that *N. virens* may be able to perform fatty acid elongation to ARA, especially as the laboratory conditions had very little alternative sources of nutrients (see Exp. 3).

Table. 36. Fatty acid composition mg g^{-1} wet weight of different experimental diets fed to N. virens: HPF = High protein fish meal, HPP = High protein plant and blood meal, LPF = Low protein fish meal, as well as organic matter sampled from water at the polychaete farm.

Fatty acid	HPF	HPP	LPF	Organic matter
14:0	1.90	0.07	0.36	0.08
16:0	6.75	5.19	4.45	1.43
18:0	1.87	1.90	1.91	0.07
16:1n-9	0.04	0.00	0.00	0.02
16:1n-7	4.10	2.07	6.79	0.29
18:1n-9	9.60	10.83	14.81	0.37
18:1n-7	1.41	0.55	0.95	0.28
20:1n-9	2.84	0.26	0.73	0.35
18:2n-6	0.81	0.91	11.50	0.14
20:2n-6	0.06	0.00	0.00	0.19
20:4n-6	0.00	0.00	0.06	0.33
20:3n-3	0.00	0.00	0.00	0.00
20:5n-3	0.13	0.00	0.53	1.34
22:1n-9	2.85	0.00	0.68	0.01
22:6n-3	0.70	0.00	1.08	0.45

Table. 37. Fatty acid composition mg g^{-1} wet weight of N. virens subjected to laboratory and farm environments when fed different diets (HPF = High protein fish meal, HPP = High protein plant and blood meal, LPF = Low protein fish meal). Mean values ± SD.

		Laboratory			Farm		
Fatty acid	**Initial**	**HPF**	**HPP**	**LPF**	**HPF**	**HPP**	**LPF**
14:0	0.24	0.29 ± 0.07	0.19 ± 0.03	0.15 ± 0.02	0.31 ± 0.03	0.30 ± 0.05	0.25 ± 0.02
16:0	3.81	4.04 ± 1.25	4.34 ± 0.34	3.25 ± 0.11	4.67 ± 0.27	4.45 ± 0.71	3.93 ± 0.67
18:0	0.61	0.74 ± 0.21	0.87 ± 0.05	0.68 ± 0.04	0.88 ± 0.01	0.78 ± 0.10	0.73 ± 0.11
16:1n-9	0.14	0.14 ± 0.04	0.14 ± 0.01	0.10 ± 0.01	0.17 ± 0.00	0.15 ± 0.03	0.13 ± 0.02
16:1n-7	1.74	1.94 ± 0.53	2.05 ± 0.20	1.73 ± 0.08	2.15 ± 0.33	1.83 ± 0.35	1.66 ± 0.32
18:1n-9	1.75	2.58 ± 0.76	3.35 ± 0.22	2.62 ± 0.06	2.50 ± 0.42	2.44 ± 0.15	2.21 ± 0.58
18:1n-7	1.85	1.94 ± 0.64	2.09 ± 0.08	1.61 ± 0.12	2.26 ± 0.03	2.13 ± 0.41	1.79 ± 0.38
20:1n-9	0.77	0.86 ± 0.25	1.02 ± 0.02	0.74 ± 0.10	1.16 ± 0.06	0.98 ± 0.14	0.88 ± 0.13
18:2n-6	1.67	1.09 ± 0.29	2.25 ± 0.27	1.33 ± 0.06	1.19 ± 0.18	1.18 ± 0.22	1.01 ± 0.42
20:2n-6	1.13	1.03 ± 0.26	1.33 ± 0.19	1.05 ± 0.10	1.07 ± 0.18	0.99 ± 0.14	0.82 ± 0.15
20:4n-6	0.40	0.34 ± 0.07	0.54 ± 0.07	0.26 ± 0.03	0.21 ± 0.05	0.14 ± 0.02	0.13 ± 0.05
20:3n-3	0.14	0.11 ± 0.03	0.15 ± 0.02	0.11 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:5n-3	1.89	1.24 ± 0.29	1.47 ± 0.12	0.87 ± 0.17	0.83 ± 0.29	0.68 ± 0.24	0.57 ± 0.26
22:1n-9	0.26	0.64 ± 0.20	0.17 ± 0.01	0.19 ± 0.04	0.45 ± 0.04	0.39 ± 0.02	0.35 ± 0.09
22:6n-3	0.73	0.56 ± 0.13	0.47 ± 0.04	0.35 ± 0.03	0.39 ± 0.08	0.36 ± 0.08	0.36 ± 0.20

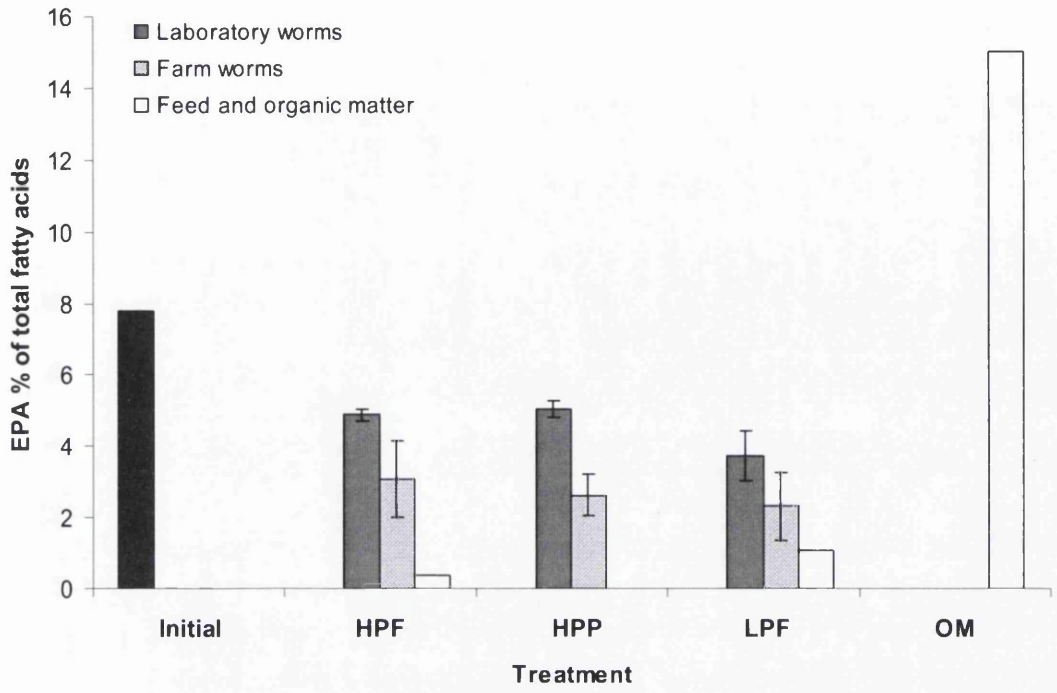

Fig. 20. Level of EPA in % total fatty acids of laboratory and farm reared N. virens relative to the amount of fatty acid in the feed (HPF = High protein fish meal, HPP = High protein plant and blood meal, LPF = Low protein fish meal), organic matter extracted from raceway water and to initial worm samples. OM = organic matter. Mean values ± SD.

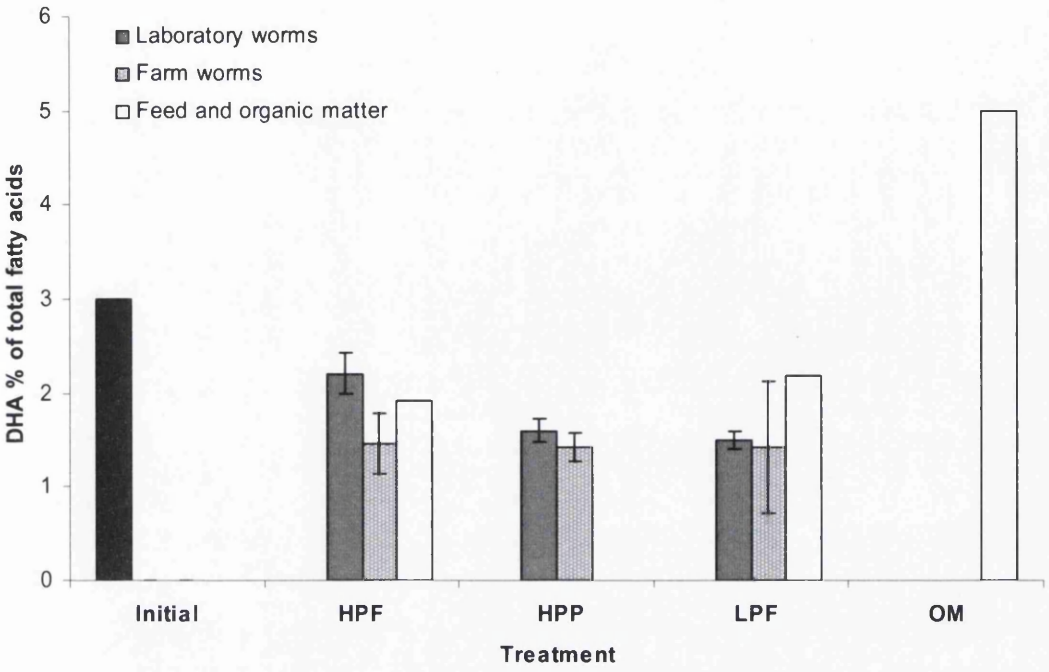

Fig. 21. Level of DHA in % total fatty acids of laboratory and farm reared N. virens relative to the amount of fatty acid in the feed (HPF = High protein fish meal, HPP = High protein plant and blood meal, LPF = Low protein fish meal), organic matter extracted from raceway water and to initial worm samples. OM = organic matter. Mean values ± SD.

6.4 Discussion

In Experiment 6, the growth response of *N. virens* to a variety of different diets was similar. All diets performed comparably with regard to survival and growth rates; SGR ranged between 1.22 and 1.61 % day^{-1} (Table 25). The general, SGR values were low compared to previous experiments (see Chap 3 and 4) although there is little research performed on alternative diets for *N. virens*. SGR of *N. virens* fed shrimp meat ranged from 4.7 to 5.9 % day^{-1} and was most efficient at salinity of 10 ‰ for worms of 0.24 – 0.8 g wet weight (Nielsen, 1995), these worms were substantially smaller than those used in the present research. Olivier (1994) demonstrated that *N. virens* grew while fed plant material; this was also seen for worms fed the soy diet in the present research.

N. virens was able to survive and grow on both plant, meat and fish based diets, as seen in both experiments 6 and 7. The findings hence confirm an ability of *N. virens* to adapt to and consume a wide variety of foodstuffs, whether of plant or animal origin, probably associated with the natural fluctuations of its food source in the wild. In a littoral system, *N. virens* has been found to consume algal detritus, other polychaetes, diatoms, hydrozoans etc...(Caron et al., 2004, Fauchald and Jumars, 1979) and also is known to be able to adapt digestive enzymes, for example, cellulase is produced in the gut when algae is available (Bock and Mayer, 1999). Lewis and Withney, 1968, found that cellulase activity was high when the worms had high algae content in the gut but not when cockles were consumed. The production of many different types of enzymes indicates that many dietary sources can be assimilated. High protein diets tested in Experiment 7, either sourced from fish or plants, resulted in higher growth rates than the low protein fish feed (high levels of protein also led to higher growth rates in Chapters 3 and 4). This effect was more clearly seen in the laboratory worms; growth rates were lower for all diets at the farm location (Table 30).

This opportunistic and adaptable feeding behaviour makes cultured *N. virens* relatively easy to provide suitable diets for while obtaining high growth and survival rates, unlike many fish and crustacean species. In penaeid shrimp, fish oils rich in n-3

and n-6 fatty acids were more efficient in terms of growth than vegetable oils poor in these fatty acids (Kumaraguru vasagam et al., 2005).

However, although growth and survival are important, the nutrient composition of the worms needs to be evaluated as it is essential if the worms are to serve as feeds in aquaculture. Protein and energy gains were similar for all diets in Experiment 6, demonstrating that the quality of the amino acid profile in plant diets was adequate for *N. virens* (Table 27). In Experiment 7, protein retention efficiency was high for the high protein diets used in the laboratory (Table 34); the low protein diet and all farm diets showed low protein retention. This trend is normal in the laboratory as the feed intake was the same for all animals.

Going by the results presented above, it would seem the best solution to feed the worms soy based diets as it is more sustainable than fish meal and is currently less costly than fish based meals. A key factor however is the fatty acid profile of the worms subjected to the different diets. The ability to obtain DHA and EPA either by elongation/desaturation of fatty acids from non marine lipids in diets or from the potential to use phytoplankton and/or microbenthos at the farm will ultimately be a decisive factor in establishing diets for *N. virens*.

Tissue DHA and EPA levels were low in diets lacking in fish meal in Experiment 6 (Table 29); this indicates that the worms cannot or very little elongate and desaturate fatty acids to n-3 essential marine fatty acids. A related suspension feeding species, *N. diversicolor*, was found to apparently biosynthesize *de novo* EPA and to a lesser extent, DHA. The levels of these acids were higher in *N. diversicolor* than in the food (Fidalgo e Costa et al., 2000).

In the current research, the positive correlation between the levels of EPA and DHA in the feed and the percentage of the fatty acids retained in the body shows that the DHA and EPA levels in the worms matches that of its feed and that it is probable that *N. virens* has a limited ability to elongate n-3 fatty acids; the worms preferentially

retain essential n-3 HUFA in its tissues. Retention of the fatty acid is more likely to occur when the fatty acid is present in the diet. When the fatty acid is lacking in the diet, there is a higher chance of an elongation step occurring, as seen in the ARA net gain of the worms for the laboratory HPP diet despite the absence of this fatty acid in the diet. The levels of EPA and DHA in the worms receiving the plant based diet in Experiment 7 were not significantly different to worms receiving fish meal diets (Table 37). The reasons for this are not clear but could be due to an ability to retain these fatty acids over a period of time in the case of laboratory reared worms or the utilization of surrounding organic material in the water and sediment within the farm. The diet fed to the worms by the industrial sponsor was very similar to the HPF diet used in the experiment, with very low levels of EPA and DHA due to the low levels of hydrolysed fish meal and high amount of plant based ingredients. The higher levels of EPA and DHA in the initial worm samples furthers the idea that the worms are not only utilising feed pellets but are also obtaining lipids from other sources.

Lytle et al., 1990, analyzed samples of wild *N. virens* (cited as *Nereis viridens*, an obsolete name for *N. virens*) and found that ARA levels were 40.6 $\mu g\ g^{-1}$ wet tissue, which is much less than the analyzed initial farmed worms in Experiment 6. ARA levels decreased to 0 for the animals fed the poultry feed diet. Some research has found ARA to be as or more important than DHA in shrimp maturation (Meunpol et al., 2005). The levels of ARA in *N. virens* were very low, between 1.04 and 1.42 % of total fatty acids in Experiment 6 (Table 29) while Lytle et al., 1990, found ARA levels of 1.3 % of total fatty acids. Meunpol et al., 2005, assumed the difference in polychaete fatty acid profiles stems from differences in habitat, environment and/or food webs, which the polychaetes inhabit.

The levels of EPA and DHA in wild *N. virens* were 25.33 % and 2.88 % of total fatty acids respectively (Lytle et al., 1990), which is a much higher level of EPA and slightly lower DHA than cultured worms (in Experiment 6). Many studies have shown that both EPA and DHA are essential fatty acids for marine fish (Sargent et al., 1999) and penaeid shrimp (Jones et al., 1979, Colvin, 1976); the levels of EPA in cultured

worms may be currently insufficient when used in diets and may need supplementation. The balance of n-3/n-6 fatty acids has also been demonstrated to be important in shrimp maturation diets (Lands, 1986) and Lytle et al., 1990, suggested that wild *N. virens* should provide an excellent balance of these fatty acids. In marine fish, DHA is needed at high levels for neural development and also the ratio of EPA:AA is important for eicosanoid actions (Sargent et al., 1999).

The levels of EPA and DHA were higher in the initial worm samples in Experiment 6 than in samples collected at the end of the experiment, including experimental worms receiving the same control feed as the farm from which the initial samples originated. This may imply that worms at the farms are utilizing an alternative feed source from their environment, possibly microalgae, protists or small invertebrates which contain EPA and DHA. Microphytobenthos may be consumed during sediment ingestion; phytoplankton and organic detritus can also be utilised from the water column during irrigation.

In Experiment 7, levels of EPA and DHA in worms analysed at the start and end were lower than those found in Experiment 6. Both were sampled from the farm prior to the experiments, however seasonality may have led to differences in fatty acid composition; Experiment 6 was carried out in July-August while Experiment 7 took place in May, there may have been a higher density of phytoplankton and other organic material present during mid-summer. The discrepancy could also have occurred due to the different trial durations; Experiment 6 lasted 56 days while Experiment 7 finished after 42 days. It should be also noted that although the initial samples from both experiments contained different levels of EPA and DHA, the HPF and LPF feeds used in Experiment 7 also had lower levels of these fatty acids than the fish meal feeds from Experiment 6, which helps to explain to the lower retention of n-3 HUFA in Experiment 7.

The variety of feeding strategies employed by *N. virens* implies that the animals made use of the algae and microzooplankton present in the water or sediment as suggested by their fatty acid profile. Luis and Passos, 1995, found that the fatty acid

composition of *N. diversicolor* reflected an omnivorous type of feed mode by sediment swallowing. The sediment was thought to be made up of microphytobenthos, mainly benthic diatoms and the fatty acids of the worms showed an accumulation of dietary fatty acids of plant origin. Colonisation of HUFA synthesizing bacteria on sediment may represent an important source of fatty acids to higher trophic levels (Müller-Navarra et al., 2000). In the case of the polychaete *Stauronereis rudolphi*, Testerman, 1972, estimated that the uptake of total long chain fatty acids could support 16 % of the worm's oxidative metabolism, yet the author estimated that contribution could be higher in areas of higher fatty acid concentrations.

In Experiment 7, a number of variables in the farm may have contributed to a decreased performance by *N. virens*. Deschênes et al., 2005, stated that relative activity in response to food stimuli in *N. virens* seemed to be controlled, first and foremost, by temperature. This implies high activity levels in warm temperatures and low activity during cold periods. The constrained environment in the farm where the worms were cultured leads to sharp temperature fluctuations. In the shallow, narrow water raceways temperatures can soar during summer months and plummet significantly during the night (16±4°C during the day, Experiment 7). These sharp fluctuations may have perhaps led to a decreased performance in terms of growth and feed intake as the worms continually adapt to changing conditions. At high temperatures, energetic costs and growth rates are high. The water quality may also have been affected by the high concentrations of phytoplankton leading to oxygen depletion. High numbers of amphipods were seen in the farm raceways in and around the experimental tubs. They were more than likely consuming feed and are contributing to less feed being available to the worms.

Calculated FCR values were unusually high for the farm ragworm, between 2.73 and 14.83. This is thought to have been due to the inability to monitor any leftover feed and the habitat in which the ragworm were trialled in. A very high number of amphipods were seen swimming in the water in and around the experimental set-up at the farm and many were consequentially found (as well as other small polychaete infauna) during experimental takedown in the sediment. The amount of feed consumed

by the amphipods was unknown and an accurate feed intake % and FCR could not be obtained.

During the course of the trial, less energy was deposited in the farm animals than in the laboratory in Experiment 7. This effect may be due to more energy being expended for burrow irrigation in an attempt to maintain optimal oxygen levels; anoxic conditions are attained rapidly and predominate in nereid burrows during resting periods (Miron et al., 1992). This condition may have been more prevalent at the farm as the mesh surrounding the experimental tubs was becoming increasingly obstructed and the only means of oxygenating the water in the raceway was a paddle aerator. The difficulties in carrying out a farm based experiment may have resulted in increase stress upon the animals as little water exchange occurred between the experimental tubs and the surrounding raceway.

To conclude, Experiments 6 and 7 demonstrate that *N. virens* is adaptable to a wide range of feeds and is capable of growth, survival and nutrient gains when fed plant or non fish meal based diets. Concerning the fatty acid profile, *N. virens* conserves levels of n-3 HUFA in the tissue and does not appear to be able to synthesise DHA or EPA from dietary precursors, the worms did however show a net gain of ARA in the tissues for the laboratory HPP diet despite the absence of this fatty acid in the diet which indicates the possibility of elongation of ARA in the body. *N. virens* is however likely to utilise organic matter in the form of phytoplankton and other microbenthos from the polychaete raceway environment in order to supplement levels of fatty acids in the body. The use of such natural resources in a polychaete farm would be a significant asset when devising sustainable non fish meal based feeds.

CHAPTER 7

General Discussion

In recent years, polychaete farming has been seen as a method of providing a marine based feed to the aquaculture industry in order to decrease the dependence of wild caught fish which are increasingly becoming depleted in the oceans. Polychaetes are a natural feed source for fish and crustaceans in the wild and contain high levels of protein and lipids. A major obstacle, however, was that the polychaetes were themselves fed marine fish based feeds which was deterring the industry's pursuit of being environmentally friendly and sustainable. The key aims of this project were to help polychaete farmers by quantifying the nutrient requirements so that feeds could be developed and tested. The lack of research and published data in this field has resulted in vague guidelines for feed requirements of *N. virens*. The worms were arbitrarily fed fish meal based diets which had a nutritional profile and ingredient composition which was not tailored to their needs. The lack of published work on feeding requirements for *N. virens* also signified that a large element of method development was needed in order to achieve the goals. The research required methods of rearing *N. virens* in the laboratory in such a way that results could be translated into tangible working techniques in the polychaete farm itself.

Various nutritional experiments were carried out with *N. virens* with the aim of optimizing rearing techniques for this species within polychaete farms. The aims of this research were to quantify the nutritional requirements, notably protein and energy, of *N. virens* at different weight classes as well as the potential of the worms to consume alternative sources of feed.

What is the optimum feeding level for *N. virens*?

A basic and important component when looking at the nutritional requirements of *N. virens* was the effects of different feed rations on the survival, growth and nutrient composition of the animals. Results demonstrated that a low feed intake of under approximately 1 % of the worm's body weight led to lower growth rates as well as lower protein and energy gains, although survival was unaffected. *N. virens* was obviously not receiving enough nutrients to cover metabolic needs, growth and nutrient

deposition. The priority of any ingested protein and energy was principally for metabolic requirements. The research also showed that there were no advantages in feeding worms to satiation. Feeding a maximum amount resulted in lower growth rates, less nutrient retention and a high FCR, as seen in many fish species such as rainbow trout, striped bass and channel catfish (Van Ham et al., 2003). The high amount of feed was not being converted efficiently in the body and was in excess of the animal's needs.

A high feeding ration of between 2 and 3 % of the worm's body weight, adjusted relative to the maximum feed intake for worms of a given size, appeared to be the most efficient feed level. A decrease in performance above this feed ration was also seen for *Nereis diversicolor* (Bischoff, 2007) and in shrimp (Kureshy and Davis, 2002). When looking at the effects of feeding a low protein diet at high quantities or a high protein diet in lower quantities, it became clear that there more advantages in using low amounts of a high protein feed. A high amount of energy was required to process large quantities of feed which led to lower energy retention.

What are the effects of starvation on *N. virens*?

Starvation studies were conducted in order to examine the effects on the metabolism and physiology on *N. virens* as well as providing data sets needed to obtain maintenance values. *N. virens* was able to withstand long periods of time without feed. Animals undergoing starvation acquired more water in the body, possibly to maintain their body weight or perhaps to increase their water intake in order to extract nutrients (Chapman and Taylor, 1968; Bock and Mayer, 1999).

The worms also had a higher ash content which resulted from a decrease in protein and lipid and hence an increase in non useable inorganic matter. There was no correlation between protein or energy loss at starvation and weight of the animals. Starved animals at the farm appear to have lost less weight than worms in the laboratory and less ash content. The worms may have been taking advantage of organic matter present in the sediment and water (Bock and Mayer, 1999; Christensen et al., 2000).

How can bioenergetic modelling help in predicting the nutritional requirements of *N. virens*?

The protein and energy requirements for *N. virens* for different weight classes were calculated using the formula: Energy/Protein requirement = a x BW (g) + c x growth. This equation was derived from Lupatsch and Kissil, 2005.

a = maintenance requirement
BW = body weight
c = cost of energy deposition (reciprocal of the slope in the nutrient consumed – nutrient gained relationship)
growth = amount of energy and protein gain respectively

The equation required knowledge of the maintenance value, the average body protein and energy content, the potential for nutrient gain as well as growth. The maintenance value for energy was found to be 18 J g^{-1} day^{-1} and for protein 0.19 mg g^{-1} day^{-1}. The average nutrient content in the body remained unchanged as the worms increased in size and was 4822 J g^{-1} for energy and 101.41 mg g^{-1} for protein. The growth potential was derived from a series of data encompassing many trials and weight classes and resulted in the equation
$y = 0.015x^{1.106}$.

From this data, a feeding table (Table 22) for *N. virens* for weights of 1 to 14 g could be derived. In this table, the daily protein and energy requirements as well as the daily weight gain and feed intake could be deduced which will allow for a precise feed delivery to worms of all weights.

What are the effects of increasing dietary protein levels on *N. virens*?

Protein requirements were investigated by feeding varying levels of dietary protein to *N. virens*. There was a linear trend to protein gain in relation to protein consumed up until a critical point at which growth and protein gain decreased. The highest protein gains and animal growth occurred for high levels of protein but not when fed to satiation. This has also been documented for many fish species such as

tilapia (Kaushik et al., 1995), trout (Kim and Kaushik, 1992) and gilthead seabream (Vergara et al., 1996). Approximately 25% of protein consumed was deposited in the body, at satiation levels the protein retention efficiency decreased. This was thought to be due to an inability to retain more protein which was therefore excreted and/or unnecessary energy expenditure to ingest and process the feed. Excess protein is also used as an energy source which is inefficient as protein is a more expensive and valuable feed component than lipid and carbohydrates.

The most efficient protein utilisation appears to be attained by feeding *N. virens* high rations of 40 to 45 % protein feed. This results in a suitable FCR of around 1.11 to 1.43, a high SGR as well as high protein and energy gains in the body. Protein content in feed influences growth in *N. virens.* Feeds may require a higher energy:protein in order to ensure that protein is not used as an energy source but instead used for growth.

What are the effects of increasing dietary energy levels on *N. virens*?

In an economical and environmental sense, lipids and carbohydrates are considered the main dietary sources of energy in *N. virens* and should be used preferentially over protein. Similarly to protein, there was a high energy retention efficiency (30.05, Table. 15.), when energy intake was low and a low retention efficiency (20.39, Table. 5.) at high energy feeding levels. The relationship between energy consumption and energy gain was also linear up to a maximum point when growth and energy gains decreased. *N. virens* was unable to retain excess energy in the body or had to expend more energy to consume high quantities of feed.

The optimum dietary energy was concluded to be at high levels, but not satiation levels. However, high energy retention efficiencies occurred for worms consuming high levels of protein; a high amount of nutrients were available for growth and metabolic activities which resulted in more energy being deposited in the body. This was also seen in the case of white seabream (Ozorio et al., 2006). A higher energy level may however be needed in feeds relative to protein in order for high levels of protein to be utilised more efficiently.

What is the potential for alternative feeds on *N. virens* culture?

Researching different feeds plays a critical part in promoting sustainable rearing of *N. virens* in order to find a replacement for fish meal based diets. Various diets based on fish meal, poultry meal and soy meal were fed to *N. virens* to look at the effects of these feeds on the growth, survival and nutritional composition of the worms.

All feeds resulted in similar levels of growth, survival as well as protein and energy gains in the body. *N. virens* is adaptable and able to consume different feeds; it has been demonstrated that this species in the wild has many different feeding strategies such as detritivory, omnivory and deposit feeding (Caron et al., 2004; Fauchald and Jumars, 1979). The different composition of feeds based on plants and terrestrial animals compared to fish meal did not lead to a decrease in performance (SGR of 1.22 to 1.61 % day^{-1}) or protein gains (1.42 to 1.93 mg g^{-1} day^{-1}) of *N. virens*, as seen in Experiment 6.

Can *N. virens* elongate fatty acids to AA, DHA and EPA?

Lipid content and fatty acid composition are important factors when considering feed sources for aquaculture species. The essential fatty acids present in *N. virens* make it well suited to serve as a feed source for crustaceans and fish; in particular fatty acids EPA and DHA which are essential for healthy development of the animals (Rainuzzo et al., 1997; Watanabe, 1982; Sargent et al., 1989). A focus of the research was an investigation into the ability of *N. virens* to retain and elongate fatty acids from non marine based feeds.

DHA (0.34 – 0.40 mg g^{-1} wet weight) and EPA (1.33 – 1.59 mg g^{-1} wet weight) levels in the body were low for diets lacking in fish meal; this shows that the worms cannot elongate n-3 fatty acids to these essential HUFA fatty acids. The results demonstrate that *N. virens* require DHA and EPA to be present in their feed source. *N. virens* however appears to be capable of retaining high levels of n-3 in the body.

Can *N. virens* utilise fatty acids from other sources?

An interesting result from Experiment 6 and 7 shows that all diets resulted in lower levels of EPA and DHA relative to the initial worm samples taken from the farm. For the control feed in Experiment 6 and the HPF feed in Experiment 7, the only difference the worms were subjected to was their environment. The farmed worms lived in an environment occupied with macro- and microfauna as well as water containing around 1 g wet weight of organic matter/algae per litre while the laboratory had very little life within the recirculating system. The high levels of EPA and DHA present in the water at the polychaete farm demonstrates the potential of ambient organic matter and microalgae as supplemental feed sources for *N. virens*.

How can this research be put to practical use?

The results can be used in the context of a ragworm farm in two main ways. Firstly, having created a feeding table, worms need not be fed arbitrarily but instead be provided with dietary formulations and feed amounts which suit their needs. This will allow for a reduction in waste feed accumulating in the environment which could lead to a degradation of the water and sediment. The reduction in waste would also lower feed costs as only the necessary feed amounts would be given each day. Secondly, the research has shown that *N. virens* is adaptable and can consume a wide range of feeds but cannot elongate short chain fatty acids to EPA and DHA. This species is however likely to be able to utilise other sources of fatty acids from organic matter in water and sediment and thus be able to supplement any formulated feeds. *N. virens* was commercially being fed a low EPA and DHA diet based on vegetable sources and hydrolysed salmon meal at the time of research; the higher levels of these acids in the body on *N. virens* demonstrates a high likelihood that other feed sources in the environment are being utilised. There does appear however to be a likelihood that *N. virens* can elongate fatty acids to AA, as seen by the net increase of this fatty acid in the worm's when fed diets lacking in AA.

Research Improvements

- Population differences

A puzzling element of the research was the different growth rates seen among the experiments. Growth rates for animals of the same initial size resulted in different growth rates. The principle variable identified to distinguish the worms between experiments was the time of sampling the worms initially. It appeared that different cohorts grew at various rates depending on the time of year in which they were spawned. The data could have been improved by conducting experiments with worms from similar birth times or by investigating more precisely how different cohorts produced different results.

- Experimental design

The research may have been improved by using individual worm data sets in order to generate a more precise and accurate statistical output. The constraints of time and space (small wet laboratory) made such research difficult; perhaps a tagging system by which individual ragworm could be identified within communal tubs may have improved the experimental design. In the absence of individual identification, it was necessary to calculate single mean values from each tub replicate, which may have reduced the statistical power of the experiments.

- Larger weight classes

The present research used worms with mean weights ranging from circa 1 to 13 g. The feeding behaviour of smaller sized worms remains unknown. By using worms with a size less than 1 g, more information on feed intake, nutrient gains and weight gains could be quantified which would allow for a more precise feeding table. However, such small worms are difficult to obtain from ragworm farms. It would have also been of interest to look at the feeding behaviour of larger worms, however the marketable

size of the worms is usually below 13 g and the current research had a focus on the common worm sizes sold and processed at the farm. Future work could also look at the feeding requirements of maturing worms. It is known that energy is focused on reproduction at this time and nutrients requirements are likely to be different, for example, lipids for egg formation. Worms also cease to feed before spawning which would again be taken into consideration as feeding a raceway with worms preparing to spawn would be wasteful.

- **Field trials**

The experiment conducted at the farm did not provide the desired accurate results due to the difficulty in conducting a semi-closed experiment within the raceway. The main problems were providing sufficient oxygen into the tubs, avoiding the presence of high amounts of fauna within the experiment and the lack of visibility to observe feed intake and mortality. The design of this experiment could perhaps have been improved by finding a system whereby the green water from the raceway could have been used without conducting the experiment within the raceway itself.

Future work

- **Practical demonstration**

The culmination of the research would be to use the results practically at the farm. An entire raceway containing an *N. virens* population of known size and density would be ideal to test the feed requirements calculated for the given weight class. The experiment could also use a soy based feed to observe the changes in fatty acid profile. It would be important to see how the laboratory results translate into working feeding strategies in a polychaete farm. Growth, survival, nutrient retention and fatty acid profiles would be analysed and compared to the present research and investigations carried out on the differences that may occur. There would be substantial difficulties in setting up such a large scale experiment such as the provision of a control raceway with

clean sediment and water, and problems similar to those encountered during Experiment 7, such as a lack of visibility into the ponds to observe feed intake, mortalities etc.

- Polychaete farm raceway ecosystem

Despite the substantive new research findings that have been generated via laboratory and field experiments, considerable additional information would be needed to accurately extrapolate to full farm scale . The amount of fauna, especially amphipods, present in the water is perhaps the greatest challenge to such research. The densities of these animals are unknown, and so are the quantities of pellets which they consume. Another unknown is the extent at which these animals provide a feed source for *N. virens* either from faeces, moults or carcasses. Further research could be spent looking at the ecosystem within the raceway and the interactions and energy fluxes between the macro- and micro-fauna present in the water column and sediment, *N. virens* and microalgae. Perhaps tackled in a food web investigation manner, this study could look at the nutrient sources within the raceways and their consumers. Although mentioned previously that excess feed may be detrimental to the environment and economically wasteful, this type of research would look at whether excess feed is perhaps needed to fertilise the ponds and provide a pool of nutrients for other organisms.

- Alternative polychaetes

N. virens is a major species in polychaete farming but not the only one. Other species being investigated with potential uses in aquaculture include the lugworm *Arenicola marina* (Olive, 1999; Scaps, 2003) and ragworm *Nereis diversicolor* (Scaps, 2002). Both these species common to British shores have been farmed, yet their own feeding requirements have yet to be quantified. *A. marina* is a deposit feeder (Riisgard and Banta, 1998), while *N. diversicolor* has been described as an omnivore but also capable of capturing food with mucus secretions (Scaps, 2002), which *N. virens* is not thought to be able to do. The differences in feeding types and potential uses in

aquaculture would make both these species worthy of investigation using similar methodologies as used in the present research.

Appendix

1. Source Data for Bioenergetic Calculations

Table. 38. Source data points from Experiments 1, 2, 3 and 5 used in Chapter 5, Fig. 16 and 17.

	Protein consumed mg g^{-1} day^{-1}	Protein gain mg g^{-1} day^{-1}	Energy consumed J g^{-1} day^{-1}	Energy gain J g^{-1} day^{-1}
Experiment 1	0.00	-0.08	0.00	-7.91
	0.00	0.01	0.00	-11.57
	0.00	-0.13	0.00	-10.72
	1.38	0.44	64.47	19.63
	1.16	0.25	58.07	6.00
	2.42	0.74	120.55	37.69
	2.27	0.93	107.62	46.96
	3.61	1.17	176.04	60.13
	3.45	1.34	168.52	63.85
	4.01	1.00	185.96	50.86
	4.28	0.98	197.61	44.71
	3.98	0.78	185.75	39.62
Experiment 2	0.00	-0.07	0.00	-7.24
	0.00	-0.23	0.00	-14.71
	0.00	-0.20	0.00	-11.77
	4.52	1.58	192.57	84.27
	3.66	1.24	155.87	65.71
	6.29	2.62	267.82	134.29
	7.07	2.08	301.00	117.08
Experiment 3	0	-0.09	0	-4.65
	0	-0.07	0	2.47
	0	-0.18	0	-6.71
	0	-0.14	0	-12.10
	0	-0.23	0	-9.26
	0	-0.11	0	-5.67
	0	-0.17	0	-18.71
	0	-0.11	0	-5.39
	0	-0.11	0	-11.96
	0	-0.17	0	-6.94
	0	-0.07	0	-4.64
	0	-0.06	0	-8.21
Experiment 5	0.00	-0.18	0.00	-9.04
	0.00	-0.28	0.00	-14.81
	0.00	-0.20	0.00	-8.86
	1.58	1.01	147.00	59.88
	1.84	1.13	171.23	72.37
	3.53	1.65	327.94	102.51
	3.01	1.51	279.02	93.61
	3.85	1.78	354.78	127.23
	3.92	1.96	464.29	171.58
	6.10	2.47	415.73	145.06
	9.16	2.70	452.44	178.57

2. Source Data for Statistical Analyses, Chap. 3, 4 and 6.

Experiment 1

Table. 39. Summary of performance results for Experiment 1 in which N. virens was fed a Dragon Research Ltd. fish meal based pellet feed at increasing rations: unfed, low, medium, high and maximum. Trial duration: 50 days. Start replicate numbers: 10 worms.

Ration treatment	Final number of worms	Average initial weight g	Average final weight g	Weight gain mg ind^{-1} day^{-1}	Feed intake mg ind^{-1} day^{-1}	Protein consumed mg g^{-1} day^{-1}	Protein gain mg g^{-1} day^{-1}	Energy consumed J g^{-1} day^{-1}	Energy gain J g^{-1} day^{-1}
Unfed	9	4.54	4.00	-10.71	0	0	-0.08	0	-7.91
	8	4.05	3.85	-4.18	0	0	0.01	0	-11.57
	10	4.64	3.96	-13.54	0	0	-0.13	0	-10.72
Low	9	4.50	5.30	16.01	16.90	1.38	0.44	64.47	19.63
	9	4.69	5.08	7.81	14.20	1.16	0.25	58.07	6.00
Medium	8	4.60	6.13	30.53	32.14	2.42	0.74	120.55	37.69
	9	4.38	5.96	31.56	28.93	2.27	0.93	107.62	46.96
High	8	4.44	6.70	45.16	49.24	3.61	1.17	176.04	60.13
	8	4.58	7.33	54.82	49.95	3.45	1.34	168.52	63.85
Maximum	8	4.66	6.77	42.21	56.25	4.01	1.00	185.96	50.86
	8	4.02	6.16	42.87	53.25	4.28	0.98	30.46	44.71
	9	4.80	6.32	30.46	54.77	3.98	0.78	185.75	39.62

Table. 40. Proximate composition per N. virens wet weight fed a Dragon Research Ltd. fish meal based pellet feed at increasing rations: unfed, low, medium, high and maximum.

Ration Treatment	Dry matter %	Protein %	Ash %	Energy J g^{-1}
Initial	18.16	9.12	1.81	4176
Unfed	19.72	9.92	2.49	4313
	19.20	9.69	2.57	4170
	19.41	9.97	2.42	4310
Low	20.30	9.79	2.27	4449
	18.43	9.65	2.21	4008
Medium	20.61	10.06	2.14	4782
	22.10	10.70	2.31	5145
High	22.43	10.82	2.29	5173
	22.44	11.03	2.55	5155
Maximum	21.20	10.42	2.02	5039
	19.77	9.89	2.16	4538
	20.88	10.34	2.37	4847

Experiment 2

Table. 41. Summary of performance results for Experiment 2 in which N. virens was fed Dragon Research Ltd. fish meal based pellet at increasing rations; unfed, low, medium, high and maximum. Trial duration: 59 days. Start replicate numbers: 12 worms.

Ration treatment	Final number of worms	Average initial weight g	Average final weight g	Weight gain mg ind^{-1} day^{-1}	Feed intake mg ind^{-1} day^{-1}	Protein consumed mg g^{-1} day^{-1}	Protein gain mg g^{-1} day^{-1}	Energy consumed J g^{-1} day^{-1}	Energy gain J g^{-1} day^{-1}
Unfed	11	2.48	2.59	2.76	0	0	-0.07	0	-7.24
	10	2.55	2.49	-1.31	0	0	-0.23	0	-14.71
	12	2.41	2.50	2.29	0	0	-0.20	0	-11.77
Low	10	2.42	4.27	47.50	34.61	4.52	1.58	192.57	84.27
	12	2.73	4.35	41.77	30.04	3.66	1.24	155.87	65.71
Medium	12	2.70	6.14	88.40	60.99	6.29	2.62	267.82	134.29
	12	2.46	5.35	74.00	61.11	7.07	2.08	301.00	117.08
High	10	2.52	6.43	100.26	102.49	10.69	2.72	454.87	150.96
	10	2.58	6.99	112.87	105.07	10.39	2.68	442.01	147.21
Maximum	11	2.50	5.18	68.61	109.15	12.74	2.18	542.12	113.56
	11	2.50	5.91	87.64	117.43	12.84	2.69	546.23	136.49
	11	2.30	6.07	96.73	125.17	14.07	2.70	598.93	145.36

Table. 42. Proximate composition per N. virens wet weight fed a Dragon Research Ltd. fish meal based pellet feed at increasing rations: unfed, low, medium, high and maximum.

Ration Treatment	Dry matter %	Protein %	Ash %	Energy J g^{-1}
Initial	18.10	9.97	2.68	3747
Unfed	17.32	9.29	2.85	3315
	17.03	9.26	3.16	3244
	16.58	8.84	3.10	3162
Low	20.44	10.29	2.36	4594
	19.36	10.07	2.24	4372
Medium	23.01	11.16	2.53	5114
	20.89	10.10	2.03	4823
High	22.42	10.54	2.26	5155
	20.80	10.06	1.82	4878
Maximum	21.69	10.74	1.99	4889
	21.57	11.03	2.07	5040
	20.92	10.24	1.92	4907

Experiment 3

Table. 43. Summary of performance results for Experiment 3 in which N. virens were starved under different environmental conditions: SSW=Sterile seawater, NSW=Unsterilized seawater, NSD=Normal sand, SSD=Sterile sand. Trial duration: 35 days. Start replicate numbers: 6 worms.

Treatment	Final number of worms	Average initial weight g	Average final weight g	Weight gain mg ind^{-1} day^{-1}	Protein gain mg g^{-1} day^{-1}	Energy gain J g^{-1} day^{-1}
SSW + NSD	6	3.78	3.37	-11.62	-0.09	-5.23
	6	4.27	3.82	-12.90	-0.07	-2.58
	6	4.21	3.69	-14.67	-0.18	-5.82
SSW + SSD	6	3.88	3.43	-12.81	-0.14	-11.74
	4	4.70	4.03	-19.19	-0.23	-9.29
	5	3.76	3.45	-8.81	-0.11	-8.39
NSW + NSD	5	3.73	3.03	-19.89	-0.17	-18.42
	6	3.73	3.15	-16.52	-0.11	-7.25
	5	4.03	3.37	-18.65	-0.11	-11.46
NSW + SSD	6	3.78	3.33	-12.81	-0.17	-6.59
	6	3.64	3.08	-16.00	-0.07	-5.75
	4	3.54	3.11	-12.48	-0.06	-5.89

Table. 44. Proximate composition per N. virens wet weight starved under different environmental conditions: SSW=Sterile seawater, NSW=Unsterilized seawater, NSD=Normal sand, SSD=Sterile sand.

Treatment	Dry matter %	Protein %	Ash %	Energy J g^{-1}
Initial	16.37	9.16	1.43	3807
SSW + NSD	17.95	9.93	1.86	4072
	18.33	10.00	1.82	4161
	17.99	9.76	1.82	4119
SSW + SSD	17.38	9.85	1.92	3867
	17.80	9.80	1.76	4090
	17.03	9.57	1.75	3840
NSW + NSD	17.89	10.62	1.89	3965
	18.78	10.44	2.05	4229
	18.00	10.51	1.97	4105
NSW + SSD	17.81	9.77	1.88	4073
	18.78	10.58	1.83	4281
	17.91	10.23	1.85	4122

Experiment 4

Table. 45. Summary of performance results for Experiment 4 in which N. virens were fed diets with increasing levels of protein, from a 15 % inclusion level to 51 %. Trial duration: 50 days. Start replicate numbers: 12 worms.

Feed protein level	Final number of worms	Average initial weight g	Average final weight g	Weight gain mg ind^{-1} day^{-1}	Feed intake mg ind^{-1} day^{-1}	Protein consumed mg g^{-1} day^{-1}	Protein gain mg g^{-1} day^{-1}	Energy consumed J g^{-1} day^{-1}	Energy gain J g^{-1} day^{-1}
15%	9	4.26	8.68	88.30	205.84	5.08	1.30	603.36	100.64
	12	4.42	9.04	92.50	164.12	3.90	1.20	462.98	116.32
	12	4.27	9.20	98.67	171.30	4.10	1.82	487.26	104.78
24%	12	4.37	8.81	88.78	157.45	6.09	1.86	467.61	104.45
	12	4.15	8.91	95.30	164.24	6.48	1.83	497.56	108.95
	11	4.35	9.35	99.95	187.36	7.05	1.38	540.80	105.11
33%	9	4.78	9.33	90.91	146.51	7.24	1.64	400.51	96.80
	11	4.40	9.49	101.83	177.01	9.04	1.80	500.04	100.53
	12	4.38	10.23	117.02	167.51	8.26	2.05	457.06	111.47
42%	12	4.51	9.83	106.48	162.93	10.28	1.77	437.65	120.09
	11	4.42	10.49	121.34	168.39	10.39	2.05	442.20	101.75
	11	4.44	10.77	126.61	172.09	10.46	2.18	445.10	118.28
51%	12	4.27	12.27	160.07	166.66	11.75	2.66	402.84	124.22
	12	4.29	11.89	152.10	160.03	11.43	2.30	391.83	128.86
	11	4.49	11.31	136.44	168.37	12.05	2.20	413.14	109.22

Table. 46. Proximate composition per N. virens wet weight fed diets with increasing levels of protein, from a 15 % inclusion level to 51 %.

Feed protein level	Dry matter %	Protein %	Ash %	Energy J g^{-1}
Initial	16.93	9.13	2.18	3650
15%	22.43	9.03	2.70	5319
	22.61	8.66	1.92	5847
	23.17	10.43	2.84	5262
24%	23.60	11.09	2.36	5488
	23.36	10.48	2.46	5415
	21.58	8.98	2.37	5285
33%	22.66	10.54	2.28	5337
	22.10	10.37	2.21	5114
	22.54	10.61	2.45	5207
42%	22.96	10.19	1.99	5739
	21.40	10.49	2.64	4842
	23.16	10.77	2.80	5301
51%	22.30	11.01	2.95	4932
	21.22	10.20	1.91	5187
	22.30	10.54	2.77	4890

Experiment 5

Table. 47. Summary of performance results for Experiment 5 in which N. virens were fed 2 diets with protein levels of 15 and 51 % at increasing rations from starvation to high. Trial duration: 43 days. Start replicate numbers: 17 worms.

Ration treatment	Final number of worms	Average initial weight g	Average final weight g	Weight gain mg ind^{-1} day^{-1}	Feed intake mg ind^{-1} day^{-1}	Protein consumed mg g^{-1} day^{-}	Protein gain mg g^{-1} day^{-1}	Energy consumed J g^{-1} day^{-1}	Energy gain J g^{-1} day^{-1}
Unfed	12	4.40	3.88	-11.96	0	0	-0.18	0	-9.04
	11	3.99	3.39	-13.93	0	0	-0.28	0	-14.81
	12	3.92	3.69	-5.25	0	0	-0.20	0	-8.86
19 % - low	12	4.66	7.11	56.86	47.46	1.58	0.72	147.00	47.03
	11	4.28	6.79	58.39	51.78	1.84	0.84	171.23	58.40
19 % - med	11	4.09	7.76	85.29	103.70	3.53	1.32	327.94	86.09
	12	3.99	9.24	122.00	95.06	3.01	1.21	279.02	78.68
19 % - high	10	4.21	10.80	153.22	222.44	6.33	1.72	587.78	97.31
	12	4.10	10.31	144.36	188.37	5.57	1.57	516.70	101.61
	11	4.11	10.99	159.98	205.24	5.86	1.76	544.10	102.35
45 % - low	12	3.91	7.92	93.16	47.46	3.85	1.44	149.18	68.31
	11	4.22	8.43	97.95	51.78	3.92	1.60	151.83	82.73
45 % - med	12	4.25	11.65	172.15	95.06	6.10	2.08	236.40	110.09
	8	4.27	11.56	169.49	142.59	9.16	2.28	354.78	109.34
45 % - high	11	4.21	13.97	226.98	203.59	11.98	2.38	464.29	127.96
	12	4.48	13.16	201.74	182.55	10.73	2.03	415.73	106.16
	12	3.69	13.98	239.32	185.76	11.68	2.68	452.44	134.14

Experiment 5

Table. 48. Proximate composition per N. virens wet weight fed 2 diets with protein levels of 15 and 51 % at increasing rations from starvation to high.

Ration treatment	Dry matter %	Protein %	Ash %	Energy J g^{-1}
Initial	16.71	10.01	1.72	3649
Unfed	17.32	10.53	2.14	3719
	17.17	10.49	2.05	3604
	16.56	9.74	2.11	3480
19 % - low	19.95	10.07	1.89	4479
	20.61	10.17	1.86	4770
19 % - med	21.52	10.44	1.97	5126
	18.45	8.59	2.52	4223
19 % - high	19.27	9.47	1.71	4485
	19.17	9.16	1.77	4674
	18.96	9.30	1.63	4508
45 % - low	18.90	10.32	1.90	4297
	20.76	10.98	1.89	4825
45 % - med	19.18	10.07	1.38	4654
	20.03	10.75	1.68	4675
45 % - high	21.29	10.80	1.69	5150
	20.89	10.62	2.06	4884
	20.95	10.69	1.85	4907

Experiment 6

Table. 49. Summary of performance results for Experiment 6 in which N. virens were fed fish meal, poultry meal and soy meal pellets manufactured at Dragon Research Ltd. as well a commercial fish meal diet used as a control. Trial duration: 56 days. Start replicate numbers: 20 worms.

Treatment	Final number of worms	Average initial weight g	Average final weight g	Weight gain mg ind^{-1} day^{-1}	Feed intake mg ind^{-1} day^{-1}	Protein consumed mg g^{-1} day^{-1}	Protein gain mg g^{-1} day^{-1}	Energy consumed J g^{-1} day^{-1}	Energy gain J g^{-1} day^{-1}
Control	14	4.05	9.97	105.79	92.85	5.69	2.39	312.23	112.57
	17	3.8	7.80	71.40	76.55	5.47	1.46	300.53	73.63
	14	3.75	9.68	105.96	89.62	5.79		317.83	
Soy meal	14	3.9	8.81	87.62	90.39	5.46	1.63	315.83	69.52
	18	3.85	7.52	65.48	71.90	4.73	1.39	273.72	62.34
	14	4.1	7.17	54.80	86.4	5.64		326.35	
Fish meal	13	4.1	10.63	116.54	99.44	6.75	1.71	288.27	78.07
	13	3.95	10.52	117.37	97.98	6.80	1.87	290.80	88.25
	19	4.0	8.62	82.54	68.71	5.24		223.87	
Poultry meal	14	3.9	9.59	101.63	91.29	6.70	1.47	332.64	75.66
	14	3.9	8.74	86.52	89.94	6.91	1.37	343.19	62.64
	16	3.9	7.97	72.65	77.39	6.23		309.35	

Table. 50. Proximate composition per N. virens wet weight fed fish meal, poultry meal and soy meal pellets manufactured at Dragon Research Ltd. as well a commercial fish meal diet used as a control.

Treatment	Dry matter %	Protein %	Ash %	Energy J g^{-1}
Initial	14.64	7.79	1.56	5840
Control	24.27	11.71	4.38	5470
	19.39	9.52	2.63	4622
Soy meal	18.84	9.53	3.13	4175
	19.66	9.58	3.69	4332
Fish meal	18.73	8.95	3.25	4097
	19.56	9.36	3.42	4371
Poultry meal	20.32	8.42	5.18	4157
	19.52	8.60	4.69	3939

Experiment 7

*Table. 51. Summary of performance results for Experiment 7 in which N. virens was fed different diets, HPF = High Protein Fish meal, High Protein Plant meal, LPF = Low Protein Fish meal as well as a unfed treatment, under both laboratory and farm conditions. Trial duration: 42 days. Start replicate numbers: 12 worms, * 6 worms.*

Treatment	Final number of worms	Average initial weight g	Average final weight g	Weight gain mg ind^{-1} day^{-1}	Feed intake mg ind^{-1} day^{-1}	Protein consumed mg g^{-1} day^{-1}	Protein gain mg g^{-1} day^{-1}	Energy consumed J g^{-1} day^{-1}	Energy gain J g^{-1} day^{-1}
Lab – HPF	10	5.16	8.08	69.31	73.81	4.80	1.12	230.36	54.60
	6	4.87	9.72	115.50	123.02	7.51	1.56	360.29	67.71
	11	4.65	7.21	60.89	67.10	4.87	0.99	233.53	48.54
Lab – HPP	11	5.49	8.54	72.72	67.10	4.12	0.99	195.61	45.47
	7	5.05	7.64	61.59	105.44	7.13	1.03	338.70	44.43
	10	5.39	7.95	60.87	73.81	4.73	0.92	224.92	41.64
Lab - LPF	5	5.48	7.99	59.87	147.62	9.37	0.62	352.30	33.94
	10	4.98	6.72	41.37	73.81	5.36	0.58	201.45	35.85
	8	5.04	5.24	4.71	92.26	7.54	0.05	283.36	6.22
Lab – Unfed	4*	5.05	4.87	-4.23	0	0	-0.17	0	-19.54
	5*	4.87	4.12	-17.64	0	0	-0.27	0	-25.35
	2*	5.32	3.55	-42.22	0	0	-1.16	0	-59.51
	9	4.81	3.92	-21.12	0	0	-0.45	0	-31.80
Farm – HPF	10	5.09	5.41	7.58	73.81	5.91	0.26	283.38	8.98
	11	4.51	5.38	20.71	67.10	5.72	0.36	274.54	12.18
	3	5.07	6.06	23.55	246.03	18.65	0.57	895.05	25.63
Farm – HPP	9	5.25	7.56	54.93	82.01	4.58	0.55	259.62	30.92
	11	5.03	6.10	25.62	67.10	4.26	0.34	241.81	18.69
	7	4.45	5.53	25.79	105.44	7.48	0.24	424.15	13.36
Farm - LPF	6	4.86	5.01	3.55	123.02	4.63	-0.08	393.49	0.39
	7	4.42	5.08	15.78	105.44	4.14	0.13	351.27	2.42
	12	4.75	5.56	19.38	61.51	2.22	0.07	188.92	6.72
Farm – Unfed	10	4.72	5.14	10.02	0	0	0.21	0	2.12
	6*	4.38	3.86	-12.22	0	0	-0.37	0	-21.85

Experiment 7

Table. 52. Proximate composition per N. virens wet weight fed different diets, HPF = High Protein Fish meal, High Protein Plant meal, LPF = Low Protein Fish meal as well as an unfed treatment, under both laboratory and farm conditions.

Treatment	Dry matter %	Protein %	Ash %	Energy J g^{-1}
Initial	18.60	9.53	1.97	4339
Lab – HPF	19.65	9.87	1.48	4609
	18.42	9.41	1.50	4187
	19.10	9.50	1.54	4437
Lab – HPP	18.67	9.45	1.56	4318
	18.89	9.81	1.50	4387
	18.80	9.64	1.54	4384
Lab - LPF	18.32	8.67	1.81	4154
	19.32	9.15	1.57	4514
	19.28	9.36	1.63	4432
Lab – Unfed	16.29	9.16	1.55	3662
	18.21	10.01	2.27	3962
	14.97	8.31	1.32	3446
	17.00	9.59	1.64	3842
Farm – HPF	19.24	10.02	1.65	4450
	17.86	9.36	1.73	4106
	19.84	10.17	1.80	4615
Farm – HPP	17.50	8.56	1.86	4098
	18.45	9.13	1.65	4286
	17.38	8.55	1.68	3993
Farm - LPF	18.46	8.92	1.60	4226
	17.76	8.80	1.60	3868
	17.48	8.42	1.79	3965
Farm – Unfed	18.07	9.59	1.74	4069
	17.58	9.15	1.62	3939

Glossary

Allochthonous = Non-native species

Biodeposit = Faeces and pseudofaeces

Bioenergetic = Field of biochemistry concerned with energy flows through living systems

Bioremediaton = Process which uses live organisms to clean up contaminated soil or water

Biosynthesis = Formation of a chemical compound by a living organism

Broodstock = Group of sexually mature individuals of a cultured species kept speared for breeding purposes

Chaetae = Bristle like projections of annelid worms.

Coelom = Fluid filled cavity within the mesoderm

Coelomocytes = Leukocyte type cell considered to be the immune cells of lower coelomate animals

Colorimetry = Measurement of the wavelength and the intensity of electromagnetic radiation in the visible region of the spectrum

Cryopreservation = Process where cells or whole tissues are preserved by cooling to low sub-zero temperatures

Curvilinear = Represented by a curved line

Embryogenesis = The development and growth of an embryo

Epitoky = Form of reproduction in polychaete worms in which the worms undergo a partial or complete transformation into a pelagic morph capable of sexual reproduction

Essential fatty acid = Fatty acids that cannot be constructed within an organism from other components by any known chemical pathways, and therefore must be obtained from the diet

Euryhaline = Ability to adapt to a wide range of salinities

Eurythermal = Ability to adapt to a wide range of temperatures

Extruder = A device that forces ductile or semisoft solids through die openings of appropriate shape to produce a continuous film, strip, or tubing

Fish solubles = Hydrolyzed fish extract

Gametogenesis = Process by which diploid or haploid precursor cells undergo cell division and differentiation to form mature haploid gametes

Germ cells = Reproductive cells in multicellular organisms

Histolysis = Breakdown and disintegration of organic tissue

Mariculture = Cultivation of marine organisms in their natural habitats, usually for commercial purposes

Nauplii = The free-swimming first stage of the larva of certain crustaceans, having an unsegmented body with three pairs of appendages and a single median eye

Neutral lipid = Uncharged lipids such as glycerides, cholesterol and cholesteryl esters

Oocyte = A cell from which an egg or ovum develops by meiosis

Parapodia = Fleshy paired appendages of polychaete annelids that function in locomotion and breathing

Phospholipid = Any of various phosphorus-containing lipids, such as lecithin and cephalin, that are composed mainly of fatty acids, a phosphate group, and a simple organic molecule

Photoperiod = Recurring cycle of light and dark periods

Polyculture = Aquaculture integrated with other agriculture activities, where wastes produced by one activity may be inputs for another activity

Prostomium = Portion of the head in earthworms and other annelids that is situated anterior to the mouth

Semalparous = Organism which dies after spawning

Spermatophore = A capsule or compact mass of spermatozoa extruded by the males of certain invertebrates and primitive vertebrates and directly transferred to the reproductive parts of the female

Sterol = Any of a group of predominantly unsaturated solid alcohols of the steroid group, such as cholesterol and ergosterol, present in the fatty tissues of plants and animals

Trochophore = Small, free-swimming, ciliated aquatic larva of various invertebrates, including certain mollusks and annelids

Vascularisation = Become vascular and have vessels that circulate fluids

Vitellogenesis = Formation of the yolk of an egg

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